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PB-295 684

Fate and Biological Effects of Cadium Introduced into Channel Microcosms

Savannah River Ecology Lab, Aiken, SC

Prepared for

Environmental Research Lab, Athens, GA

Apr 79

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Athens GA 30605

EPA-600/3-79-039
April 1979

Research and Development

PB 295684

EPA

Fate and Biological Effects of Cadmium Introduced into Channel Microcosms

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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/3-79-039

2.

3. RECIPIENT'S ACCESSION NO.
PB295684

4. TITLE AND SUBTITLE
Fate and Biological Effects of Cadmium Introduced into Channel Microcosms

5. REPORT DATE
April 1979 issuing date

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
J. P. Giesy, Jr., H. J. Kania, J. W. Bowling, R. L. Knight, S. Mashburn, and S. Clarkin

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Savannah River Ecology Laboratory
University of Georgia
Aiken, South Carolina 29801

10. PROGRAM ELEMENT NO.
1HE775

11. CONTRACT/GRANT NO.
IAG-D6-0369-1

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory--Athens, GA
Office of Research and Development
U.S. Environmental Protection Agency
Athens, Georgia 30605

13. TYPE OF REPORT AND PERIOD COVERED
Final, 5/75-5/78

14. SPONSORING AGENCY CODE
EPA/600/01

15. SUPPLEMENTARY NOTES

16. ABSTRACT

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Cadmium inputs and outputs equilibrated within approximately 20 days of initial cadmium inputs. However, approximately 20% of the cadmium leaving the channels was associated with particulates. Community components accumulated cadmium proportional to cadmium exposure levels. Cadmium was rapidly eliminated from all biotic components, with concentrations returning to levels similar to those in control channels within a few weeks in the aufwuchs community to a few months in macrophytes. Organic headpool sediments showed no significant decrease in cadmium content six months after cessation of cadmium inputs, indicating that the abiotic half time for contaminated environments is very long. Half times for elimination from channel sediments were 72 and 38 days for 5 and 10 μg/1 inputs, respectively, after Cd inputs were terminated.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Cadmium Biology Water pollution	Biological effects	06C 48G 68D

18. DISTRIBUTION STATEMENT
RELEASE TO PUBLIC

19. SECURITY CLASS *(This Report)*
UNCLASSIFIED

20. SECURITY CLASS *(This page)*
UNCLASSIFIED

21. NO. OF PAGES
173

22. PRICE
PCA08 / MF A01

☆ U. S. GOVERNMENT PRINTING OFFICE: 1979 — 657-060/1637

EPA-600/3-79-039
April 1979

FATE AND BIOLOGICAL EFFECTS OF CADMIUM INTRODUCED INTO CHANNEL MICROCOSMS

by

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Interagency Agreement
IAG-D6-0369-1

between

U. S. Environmental Protection Agency
and
U. S. Department of Energy

Project Officer

Harvey W. Holm
Environmental Systems Branch
Environmental Research Laboratory
Athens, Georgia 30605

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30605

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FOREWORD

Environmental protection efforts are increasingly directed towards preventing adverse health and ecological effects associated with specific compounds of natural or human origin. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Environmental Systems Branch studies complexes of environmental processes that control the transport, transformation, degradation, and impact of pollutants or other materials in soil and water and assesses environmental factors that affect water quality.

Environmental concentrations of cadmium, which is known to be acutely and chronically toxic to plants and animals, have increased significantly since 1945 as a result of its widespread use in many industrial processes and products. Efforts to limit human exposure must rest on a good understanding of the cycling of the metal in fresh water, estuary, and marine ecosystems and biota before its significance to water pollution can be assessed. Although many studies have been conducted on the uptake of cadmium by organisms in the laboratory, few studies have been made in complex environments. This report describes the results of a study of the fate and biological effects of chronic concentrations of the metal over a number of trophic levels during the entire growing season in a complex, artificial aquatic ecosystem.

David W. Duttweiler
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Athens, Georgia

ABSTRACT

Cadmium was continuously input to aquatic microcosm channels resulting in concentrations of 5 and 10 μg Cd/l. Cadmium accumulation into both biotic and abiotic components was determined. Biological effects of cadmium were determined by monitoring structural and functional properties of the entire system as well as structual changes in populations and compared to control systems, which received no cadmium.

Cadmium inputs and outputs equilibrated within approximately 20 days of initial cadmium inputs. However, approximately 20% of the cadmium leaving the channels was associated with particulates. Community components accumulated cadmium proportional to cadmium exposure levels. Equilibrium Cd concentrations of sediments, periphyton, macrophytes, chironomids and mosquito fish exposed to 10 μg Cd/l were 0.59, 55, 250, 40, and 40 μg Cd/g dry weight. Cadmium was rapidly eliminated from all biotic components, with concentrations returning to levels similar to those in control channels within a few weeks in the aufwuchs community to a few months in macrophytes. Organic headpool sediments showed no significant decrease in cadmium content six months after cessation of cadmium inputs, indicating that the abiotic half time for contaminated environments is very long. Half times for elimination from channel sediments were 72 and 38 days for 5 and 10 μg/l inputs, respectively after Cd inputs were terminated.

Cadmium caused significant changes in both community structure and function. Some protozoan, crustacean and insect taxa were completely eliminated from channels receiving cadmium. Other taxa showed increased or decreased relative densities. Both macrophyte and periphyton growth was inhibited by these levels of cadmium exposure. Population and community recovery was rapid, with communities with rapid growth and invasion potentials indistinguishable from control systems within weeks of the time cadmium inputs were stopped.

Methods of perturbation assessment at both the population and system level were compared. Effects can be demonstrated at both levels of organization, and system level parameters were sensitive to cadmium-induced changes, however, measurements of system level parameters were not helpful in determining mechanisms of the cadmium effects.

Microcosms of the scale and complexity studied here are useful for validating and verifying predictive models of the the fates of contaminants and testing assessment strategies but are not appropriate for toxicity testing, or determining mechanisms and coefficients of uptake, elimination or degradation.

This report was submitted in fulfillment of Interagency Agreement No. IAG-D6-0369-1 by the Savannah River Ecology Laboratory under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period May 12, 1975, to May 31, 1978, and work was completed as of May 31, 1978.

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ACKNOWLEDGEMENTS

We wish to thank the staff of the EPA's Environmental Research Laboratory, Athens, Georgia, and the University of Georgia's Savannah River Ecology Laboratory for their assistance. Special recognition is due Drs. Harvey Holm and Ray Lassiter of the EPA for their help in all phases of the study. J. Cheatham, S. Giddings and R. Didgeon provided technical assistance throughout the study. Ms. J. Coleman prepared ink drawings.

The channels, which were constructed with funds provided by the Environmental Protection Agency in 1970, have been operated by personnel of the Savannah River Ecology Laboratory since that time (Kania and Beyers, 1974; Kania _et_ _al_., 1976). Operating funds have been provided by the EPA; the DOE and its predecessors have provided office space and laboratory facilities, and a variety of crucial services.

SECTION I

INTRODUCTION

Cadmium is a relatively rare element, not found in a pure state in the environment (Hiatt and Huff, 1975), making up an average of less than one-half gram per ton of the earth's crust (Page and Berggen, 1973; Fassett, 1974). While the natural occurrence of Cd in the environment is quite small, cadmium is used in many industrial processes and products such as storage batteries, pigments, semiconductors, plastics, stabilizer compunds, alloys and plating solutions and occurs as a contaminant in zinc ores, automobile tire dust, fossil fuels and agricultural fertilizers (Friberg et al., 1971; Anon., 1975; Hiatt and Huff, 1975).

Because Cd is a trace contaminant in so many materials and is released from so many diffuse sources, reduction or elimination of point source releases may not significantly reduce the trend of increased Cd mobilization with increased general human activity. The special difficulty with metals such as Cd is their persistence. Unlike organic contaminants, metals do not degrade in the environment and regardless of their source, most metallic wastes eventually end up in surface and subsurface waters (Buhler, 1971).

Cadmium is a biologically nonessential element (Anon, 1971; Fassett, 1974; Rosenthal and Sperling, 1974) and is known to be acutely and chronically toxic to plants and animals (Lagerwerff and Spect, 1970; Flick et al., 1971; Burkitt et al., 1971; Cheremisinoff and Habib, 1971; Schroeder, 1974; Hiatt and Huff, 1975; Chadwick, 1976; Giesy et al., 1977). The human health aspects of acute and chronic Cd poisoning in humans have been reviewed extensively (Anon., 1971; Fassett, 1974; Page and Bingham, 1973; Piscator, 1974; Schroeder, 1974; Friberg and Kjellstron, 1975; Fulkerson, 1975; Hiatt and Huff, 1975; Anon., 1975; Perry et al., 1976). Beside the often cited acute "itai itai disease", cadmium has been implicated as a possible carcinogen and mutagen and Cd exposure is correlated with cardiovascular disease, renal disfunction and hypertension (Flick et al., 1971; Perry et al., 1976). Cadmium releases to the environment have increased drastically since 1945, with a concomitant increase in the reported cases of Cd toxicity.

It is difficult to set standards for human exposure because the toxic effects of Cd exposure are cumulative. At birth, human beings have essentially no Cd in their tissues and gradually and continuously accumulate Cd, particularly in red blood cells, kidney, liver, bone, pancreas and liver (Wagner, 1971). Intake by humans is approximately 200-300 μg Cd/day which accumulates at a rate of approximately 3 μg/day and is eliminated very slowly from the body (Hiatt and Huff, 1975). There is considerable evidence that organisms, which have evolved under conditions of very low Cd exposure, deal with Cd by sequestration, rather than excretion and elimination. In fact, a nonspecific metal binding protein, thionein, is present in the organs of many animals, including humans (Hiatt and Huff, 1975; Anon, 1975). It is unknown whether protection against Cd toxicity by thionein is limited or whether protection is a function of accumulation rate. Rapid increases in Cd mobilization to the biosphere, relative to geologic-evolutionary time may have severe effects on organisms. The joint FAO/WHO Expert Committee on Food Additives has concluded the "present day levels of cadmium in the human kidney should not be allowed to rise further (Anon, 1975).

To achieve this goal, a good understanding of the geologic and biotic cycling of Cd will be necessary. More work is needed on the cycling of cadmium in fresh water, estuary and marine ecosystems before the significance of water pollution can be assessed relative to both ecological effects and man's food (Fulkerson, 1975). Fleischer et al. (1964) state, after a review of the literature describing the levels of Cd in plants and animals, that "Experimental studies of uptake over the lifetime of experimental animals are required for a number of representative species and at least one food chain study should be made in each of the three environments: terrestrial, freshwater and marine. Model ecosystems (Microcosms) might be the most appropriate systems for these studies." These same authors conclude, after a very short review of the literature on ecological effects of cadmium that "Our ignorance of the effects of cadmium in natural or polluted systems is almost total."

While many studies have been conducted on acute toxic effects of Cd on and uptake by organisms and specific physiological responses in the laboratory, few studies have been conducted in complex environments. When conducted in complex systems, studies have generally addressed effects or uptake independently, focusing on single populations or taxonomic groups, under conditions such that the source term is not known and have not generally been conducted over a sufficiently long period of time to be meaningful.

Research conducted in outdoor artificial stream channels can provide a vital link between laboratory studies carried out under carefully controlled conditions and field studies which can seldom be adequately controlled or replicated. Artificial streams provide realistically complex biological systems where replication is possible, a number of treatments can be investigated simultaneously, certain critical parameters can be readily controlled, and the addition and removal of stresses can be readily effected resulting in little or no environmental damage. Microcosms can be effectively used to study both environmental transport (Draggan, 1976) and effects of toxic materials in aquatic microcosms (Taub, 1976). The microcosms used in this study were complex, self perpetuating functioning ecosystems, functionally analogous to the littoral zone of softwater paludal systems.

This study was conducted to examine the fates and biological effects of chronic Cd concentrations (5 and 10 µg/l) over a number of trophic levels, during an entire growing season in a complex aquatic ecosystem. Cadmium uptake and elimination and compartmentalization by the aufwuchs, macrophyte, macroinvertebrate, fish sediment, and water components were measured and models of Cd dynamics in these systems proposed. Rate of Cd elimination from the contaminated system was also monitored. Biological effects were measured

at the population, community and ecosystem level. A comparison of the relative sensitivity of each of these organizational levels to Cd-induced stress is presented. Effects on macrophytes, aufwuchs, microinvertebrates, macroinvertebrates, and fish populations and system level measures of Cd induced effects were made and rate of recovery assessed. As well as basic information on chronic Cd effects.

SECTION II

CONCLUSIONS

1) When exposed to 5 or 10 μg Cd/l sediments and all biologic components monitored accumulated cadmium. Cadmium accumulation was approximately porportional to exposure concentration. Equilibrium Cd concentrations of sediments, periphyton, macrophytes, chironomids and mosquito fish exposed to 10 μg Cd/l were 0.59, 55, 250, 40 and 40 μg Cd/g dry weight, respectively.

2) Cadmium concentrations in biotic components reached equilibrium within 20 days. Cadmium accumulation in the aufwuchs and fish components could be described by a model of the form $C = C_o (1 - e^{-KT})$. Biological elimination was rapid, with cadmium concentrations in the aufwuchs and macroinvertebrate communities indistinguishable from background within 30 days of cessation of cadmium exposure. Cadmium elimination from sediments was much slower. Six months after Cd inputs were terminated there was no significant decrease in Cd concentrations in organic headpool sediments. Half times for elimination from sand substrata and detritus in the channels were 72 and 38 days for the 5 and 10 μg Cd/l treatments respectively.

3) Cadmium caused effects at both the population and system levels. Standing crops of both aufwuchs and macrophytes were depressed during the time of cadmium exposure. A number of invertebrate taxa were eliminated due to cadmium inputs, while others were released from competitive or predatory influence and did well in systems receiving cadmium. Both 5 and 10 μg Cd/l treatments were chronically toxic to crayfish and snails.

4) Invertebrate and algal population structure returned to background levels within a few weeks of cadmium cessation, while fish and macrophyte populations did not recover as rapidly. This is due to the more rapid growth and colonization rates of algae and invertebrates.

5) Systems level structure and function were affected by cadmium. Algal and macroinvertebrate species diversity, leaf litter decomposition, net and gross production, community respiration, and P:R ratios were depressed due to cadmium exposure.

6) Microcosms of the scale and complexity studied here are not appropriate for toxicity testing or determining mechanisms and coefficients of uptake, elimination or degradation.

7) Microcosms of the scale and complexity studied here are appropriate for verification and validation of predictive models of local fates of trace contaminants but not of worldwide transport models.

8) Comparison of both population and system levels of perturbation assessment were composed. Effects can be demonstrated at both levels of organization and system level parameters were sensitive to cadmium induced changes, however, measurements of system level parameters were not useful in determining mechanisms of cadmium induced effects.

SECTION III

RECOMMENDATIONS

1) Results of recent studies of cadmium toxicity to microcrustaceans (Geisey *et al.*, 1977) should be considered when water quality criteria are revised.

2) Exposure of aquatic sediments to even low concentrations of cadmium results in elevated cadmium concentration in this component which is persistent and should be avoided.

3) Assessment of cadmium induced perturbations should not be monitored solely by either structural or functional attributes of systems or populations.

4) Microcosms of the type studied here are not appropriate for 1) determining uptake mechanisms or coefficients, 2) acute or chronic toxicity testing, 3) screening possibly hazardous chemicals.

5) Microcosms of the type studied here may be used to validate and verify models of trace contaminant transport and possible complex community interactions.

SECTION IV

FACILITY DESCRIPTION

The microcosm facility used in this study is located on the Department of Energy's (DOE) Savannah River Plant (SRP), a 507 km^2 reserve, including portions of Aiken, Barnwell and Allendale Counties in South Carolina, U.S.A.

The facility consists of six concrete block channels (Fig. 1) 91.5 m long, 0.61 m wide and 0.31 m deep with concrete pools (1.5 m x 3.0 m x 0.92 m) at both ends of each channel supported by a concrete slab oriented on an east-west foundation. For this study, the pools and channels were lined with a 0.05 cm thick black, polyvinyl chloride (PVC) film.

Washed quartz sand was distributed in the channels to a uniform depth of 0.05m, and a 8-10 cm layer of natural stream bed sediment obtained locally was distributed in the pools. This resulted in a system similar to local aquatic systems which have both sand and silt bank substrata (Table 1).

Water for the channels was pumped from a well located near the facility and a hydrated lime slurry was continuously pumped into the main water distribution system throughout the period of the study to produce inorganic water quality similar to that of local upper coastal plain surface waters. A single batch of lime was used throughout the study and an analysis of the treatment water is given in Table 2.

Flows were monitored by V-notch weirs on each head pool where water entered the channel. A flow rate of 95 l/min was maintained manually by an input valve located at each head pool, resulting in a water depth of 20 cm in the channels. The mean water retention time and current were 2 hr and 1.3×10^{-2} m/s, respectively.

At the time water flow was commenced, the systems were seeded with material saved from the control channels of a previous study (Kania _et_ _al_., 1976) to rapidly establish biological communities known to be well adapted to channel conditions. The macrophytic angiosperm (_Juncus_ _diffusissimus_) a species which had naturally colonized the channels during previous studies, was transplanted to the systems. Selected macroinvertebrates such as clams and crayfish were added to the systems after some growth had occurred. These organisms will be mentioned in the appropriate sections of the report that follow. End screens with a mesh opening of 4 mm were placed between the channels and tail pools to contain the larger organisms.

Cadmium inputs into four of the six channels were begun on 18 March 1976. Cadmium (as $CdCl_2$) solutions were metered into the turbulent region of the head pools where the water entered, using peristaltic tubing pumps (Sage

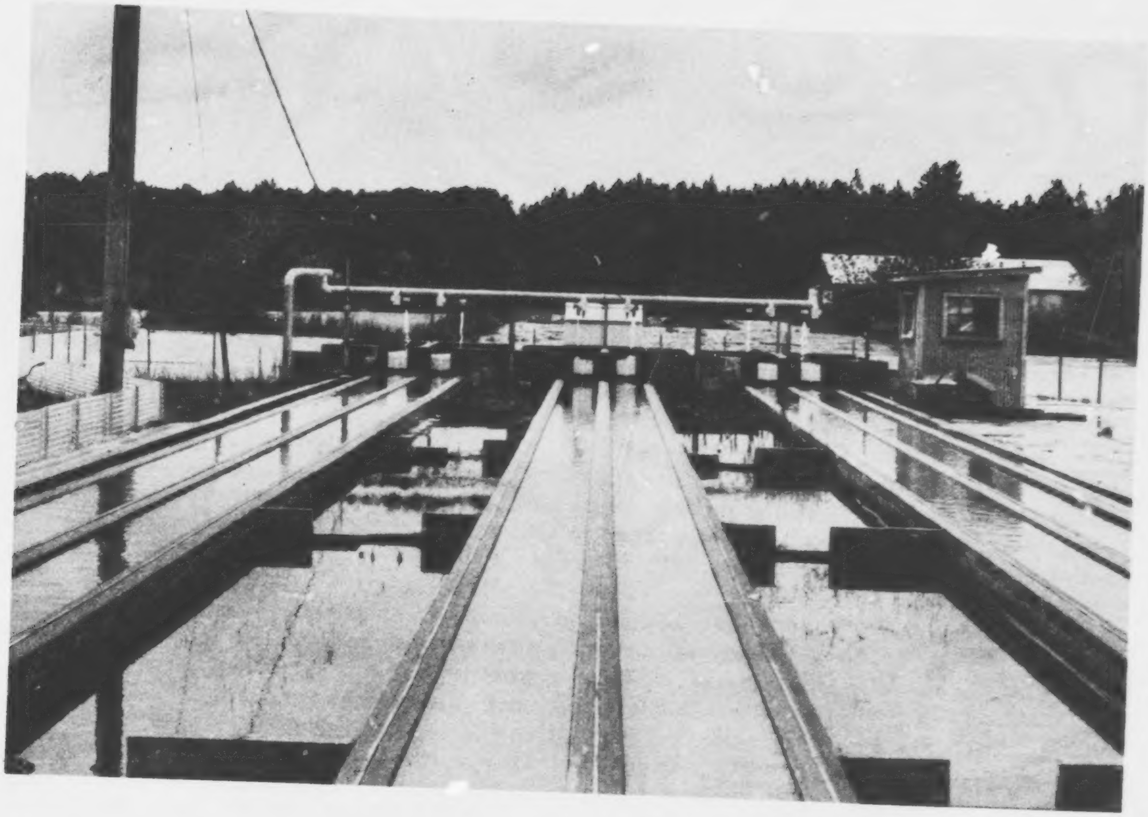


Figure 1. Stream microcosm facility.

TABLE 1. CHARACTERISTICS OF MICROCOSM SEDIMENTS

	Location		
	Head Pools	Tail Pools	Channels
Sand	37.2%	36.5%	99.8%
Silt	30.7	16.7	<0.1
Clay	3.6	12.8	<0.1
Organic Matter	28.4	34.3	<0.1
CEC	67.0 meq/100g	75.6 meq/100g	0.02 meq/100g

TABLE 2. MEAN WATER QUALITY OF TREATED WELL WATER

Total alkalinity	9.14 mg/ℓ as $CaCO_3$
Hardness	11.08 mg/ℓ as $CaCo_3$
pH	6.5
Specific conductance	31 μ mho/cm
Ionic strength (I)	2.5 x 10 - 4
Total dissolved solids	20.5 mg/ℓ
SO_4^{-2}	1.9 mg/ℓ
Total P	2.9 μg/ℓ
Nitrogen (NO_2 + NO_3)	15.8 μg/ℓ
Ca	03.17 mg/ℓ
Cu	3.4 μg/ℓ
Co	2.5 μg/ℓ
Cd	0.023 μg/ℓ
Cr	0.3 μg/ℓ
Fe	1.7 μg/ℓ
K	1.1 μg/ℓ
Mg	246 μg/ω
Mn	7.0 μg/ℓ
Na	1.8 mg/ℓ

Instruments Model 375A). Cadmium input solutions for each channel were made every two days with concentrations adjusted to compensate for pump variation over time. The Cd levels established were 5 μg/l in two channels and 10 μg/l in another two. Since the six channels are structured in three pairs (Figure 1), the dosing arrangement was chosen so that there was both a northern and a southern exposed channel for each treatment. Cadmium inputs were discontinued on 18 March 1977, one full year after they were begun.

SECTION V

WATER CHEMISTRY

INTRODUCTION

The physical and chemical state of trace metals is dependent upon water quality and must be considered when availability and toxicity of metals to aquatic organisms are assessed (McKee and Wolf, 1963; Hartung, 1973; Brown *et al*., 1974; Clubb *et al*., 1975). Metal toxicity to aquatic organisms is hardness dependent (Sprague, 1969). For example, water hardness has an antagonistic effect on Cd toxicity to zooplankton due to Ca and Mg (McKee and Wolfe, 1963). Similarly, Pickering and Henderson (1966) found increases in Cd 96 h LC_{50} values with increasing water hardness for all fish tested and Kinkade and Erdman (1975) reported that organisms accumulate Cd faster from soft than hard water. The free divalent metal ion is generally the most toxic form (Stiff, 1971; Brown *et al*., 1974; Pagenkopf *et al*., 1974). The soft acidic waters of the southeastern United States have low inorganic ligand concentrations. Thus, inorganic solubility product chemistry predicts that Cd introduced into these waters would exist mainly as free divalent cations (Cd^{+2}) or as hydrated ions ($CdOH^{+}$, $B_1 = 1.5 \times 10^{4}$; CdO_2^{-2}, $B_4 = 5.8 \times 10^{8}$) Weber and Posselt, 1974). All reactions are rapid resulting in replenishment as a particular ionic form is depleted. Giesy *et al*. (1977) found very low LC_{50} values for zooplankton exposed to Cd in the well water studied here.

METHODS

Water samples were collected monthly from each channel at a single station, located 60 m downstream of the input weirs. Temperature was measured with a YSI model 44 TD Telethermometer, conductivity with a Beckman Model RC19 Conductivity Bridge, and pH with a Orion Model 401 Specific Ion Meter with a glass electrode. Alkalinity and hardness were measured using standard EPA (1976) or APHA (1976) techniques.

Total phosphorus, nitrite and nitrate nitrogen, sulphate ion, inorganic and organic carbon analyses were performed periodically on water samples collected from the inputs and upstream and downstream locations in each channel. Downstream stations were sampled two hours after upstream and downstream locations so that the same water mass was examined and changes could be related to the biological activities in the channels.

Both organic and inorganic carbon analyses were made using a Beckman Model 915 total organic carbon analyzer. Other chemical parameters were measured using accepted EPA methods (1976) with the addition of a 5X concentration step prior to PO_4^{-3} and Cl^{-} analyses.

Samples for Cd analyses were taken from inputs to the head pools, outflows of the headpools, outflows to the channels, and outflows from the tail pools, on a monthly basis. A more frequent sampling schedule was initiated at the time the Cd inputs were terminated. Water samples were taken in 160 ml glass milk dilution bottles, which were used only for water sampling and always for the same station. After each sample was mixed well, 10 mls were transferred volumetrically to an acid rinsed polyethylene bottle and acidified with 200 μl of redistilled conc. NHO_3. A portion of the remaining sample was filtered, and 10 mls of the filtrate transferred to acid rinsed bottles and acidified. Details of the analytical procedures are given in Appendix I.

An attempt was made to separate particulate and dissolved Cd by both membrane and fiberglass filtration. Results of analyses on filtered samples were in general the same or higher than unfiltered samples. A low level contamination problem causing higher levels on filtered samples was never completely resolved.

Samples of the PVC plastic used to line the channels were suspended at both ends of each channel to see if this material either adsorbed or released into the water. Subsamples of these suspended sheets were periodically removed, washed free of periphyton, dissolved in hot concentrated H_2SO_4, oxidized with concentrated HNO_3 and hydrogen peroxide and analyzed using standard flame AA techniques. There was no measurable uptake or loss of Cd from the PVC film.

RESULTS AND DISCUSSION

Additions of hydrated lime satisfied the CO_2 demand of the well water and resulted in water similar to surface waters of the upper coastal plain (Table 2). The ionic strength of treated well water was 2.5×10^{-4} resulting in an activity coefficient for Cd of 0.97, using the extended Deby-Huckle equation. Chloride ion concentrations remained constant at all stations (Table 3). Sulphate levels increased between the upstream and downstream stations. Total organic carbon levels were below detection limits at all times at all stations. Total inorganic carbon levels decreased along the length of the channels with no differences due to Cd. Total phosphorus concentrations were low but constant at all positions, and there was a marked reduction in NO_2-NO_3 nitrogen level in both the head pools and the channels. There were no effects on nitrogen concentrations due to Cd. However, considering only data from the months of June and July, 1976, the average nitrogen uptake in the control channels was 9.6 ± 0.3 μg/l compared to 4.3 ± 1 μg/l in the 5 μ/l treatment and 5.0 ± 1 μg/l in the 10 μ/l treatment.

The observed Cd concentrations were not significantly different from the desired concentrations and there were no significant differences between sampling stations within each treatment system (Table 4). After Cd inputs were terminated, Cd concentrations in water quickly dropped and within four days were in the range of the control channels (0.02 - 0.06 μg Cd/1) at all stations. Cadmium is used as a plastisizer in PVC plastic so this material has significant concentrations of Cd in it. However, there was no measurable addition or removal of Cd due to the PVC film liner.

TABLE 3. MEAN VALUES ($\bar{X} \pm SE$) FOR CHEMICAL PARAMETERS MEASURED AT TWO STATIONS IN EACH CHANNEL DURING THE PERIOD OF CADMIUM INPUTS. SAMPLE SIZE IS REPORTED IN PARENTHESES

		Control		5 μg/ℓ		10 μg/ℓ	
		Up	Down	Up	Down	Up	Down
Cl^- (6)	mg/ℓ	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
$SO_4^=$ (12)	mg/ℓ	1.87 ± 0.08	2.10 ± 0.06	1.81 ± 0.05	2.13 ± 0.05	1.79 ± 0.07	2.19 ± 0.06
TOC (12)	mg/ℓ	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
TIC (12)	mg/ℓ	7.1 ± 0.1	4.5 ± 0.2	6.6 ± 0.2	4.4 ± 0.2	6.8 ± 0.2	4.5 ± 0.2
Total P (16)	μg/ℓ	3.5 ± 0.3	3.5 ± 0.3	3.1 ± 0.2	4.1 ± 0.5	3.3 ± 0.2	3.5 ± 0.3
NO_2-NO_3N (10)	μg/ℓ	10.4 ± 0.5	3.6 ± 1.0	8.9 ± 0.7	5.2 ± 0.6	9.3 ± 0.6	4.7 ± 0.6

TABLE 4. MEAN CADMIUM CONCENTRATIONS IN UNFILTERED WATER SAMPLES ($\bar{X}$ ± SD, n = 10)

Sampling station	NOMINAL TREATMENT 5 µg Cd/l		10 µg Cd/l	
Input	4.75 ± 2.07	5.00 ± 1.91	9.36 ± 4.22	10.15 ± 5.72
Head pool weir	4.30 ± 1.92	4.17 ± 1.85	9.45 ± 4.27	9.63 ± 5.21
Channel outflow	4.12 ± 1.71	3.94 ± 2.20	8.76 ± 3.82	9.61 ± 4.01
Tail pool outflow	4.27 ± 1.82	3.99 ± 2.26	8.30 ± 3.91	10.06 ± 4.15

SECTION VI

SEDIMENTS

INTRODUCTION

In aquatic systems, the sediments can act as both a sink for pollutants and as a source for the release of these pollutants under appropriate conditions. The behavior of cadmium in sediments and at the sediment-water interface must be known if the cycling of the element in the aquatic environment is to be understood. The purpose of the sediment work in this study was to compare the uptake and release of cadmium from two different sediment types receiving chronic known exposures to dissolved cadmium.

METHODS

Sediment samples were taken monthly from upstream and downstream stations in each channel and also from both head and tail pools. Cores were used for Cd determinations and also, in the case of sediment from the channels, to derive an estimate of organic content. Analytical procedures are presented in Appendix I. Several different sample collection techniques were used. The consistency of the highly organic silty streams sediments placed in the pools was such that cores could not be taken and sampling was done with a large syringe-like device which caused mixing of the sediments with a small amount of water when the plunger was withdrawn. Several sizes of coring tubes were used for the sandy sediments of the channels, initially, frozen cores were taken so that stratification of the cadmium within the sediment could be determined. This procedure was abandoned when it became evident that, unlike mercury (Kania et al., 1976), Cd was distributed through-out the sediment although more highly concentrated in the highly organic upper layer.

RESULTS AND DISCUSSION

The first sediment samples were taken at the end of April 1976, approximately 40 days after Cd inputs to the channels were initiated. At that time, the organic content of the channel sediments had already reached equilibrium values which were unrelated to treatment or position, and remained unchanged throughout the remainder of the study (Table 5).

Sediment Cd concentrations were at equilibrium and no further increase in levels with time were observed at any station. This is consistent with the findings of Huckabee and Blaylock (1974) who found, working with spiked microcosums, maximum sediment Cd activity was reached after only two days. Bunzl (1975) found that humic acids sorbed Cd with a half time

TABLE 5. MEAN ORGANIC CONTENT OF CHANNEL SEDIMENTS DURING STUDY PERIOD ($\bar{x}$ ± SD, n = 26)

TREATMENT	% ORGANIC
control	0.50 ± 0.36
5 μg Cd/ℓ	0.43 ± 0.10
10 μg Cd/ℓ	0.41 ± 0.11

of approximately 30 seconds, indicating that this step would not be limiting in uptake of Cd by our highly organic pool sediments. There were no significant differences between upstream and downstream stations in the channels, however, the levels in the tailpools were generally higher than those in the head pools (Table 6). Cadmium concentrations in channel sediments (Table 7) are only for the time period during cadmium input, since Cd levels in these sand sediments decreased after the inputs were terminated with mean half times of 72 and 38 days in the 5 μg/ℓ and 10 μg/ℓ treatments respectively. No significant decrease in sediment Cd concentrations of pool sediments were observed after Cd inputs were terminated so measured Cd concentrations of these sediments include samples taken during the 9 months following Cd input termination.

Naturally occurring Cd was measured in both sand and organic sediments (Tables 6 and 7). The highly organic (25-30% by weight as C) silty stream sediments had much higher Cd concentrations than the sand. The organic sedi-

TABLE 6. MEAN CD CONCENTRATIONS IN POOL SEDIMENTS ($\bar{X}$ ± SE, n = 16)

TREATMENT	Cd CONCENTRATION μg Cd/g dry weight Head	Tail
control	1.27 ± 0.10	1.50 ± 0.10
5μg Cd/ℓ	8.33 ± 0.96	21.4 ± 1.8
10 μg Cd/ℓ	10.6 ± 1.6	22.3 ± 2.7

TABLE 7. MEAN CD CONCENTRATIONS IN CHANNEL SEDIMENTS DURING CD EXPOSURE PERIOD ($\bar{X}$ ± SE, n = 18)

TREATMENT	CD CONCENTRATION (μg Cd/g dry weight)
control	0.014 ± 0.003
5 μg Cd/ℓ	0.209 ± 0.019
10 μg Cd/ℓ	0.591 ± 0.061

ments also accumulated much higher Cd concentrations when exposed to Cd. Cd uptake by sand was linearly proportional to Cd exposure, while that of organic silt sediments was not. Tail pool sediments acquired greater concentrations than did that in head pools.

Cadmium concentrations in control channels are in the range of values reported by other investigators for background levels (Table 7). Fleisher et al. (1974) reported on average value for 26 samples of unspecified lake sediments of 11 μg/g. Forstner (197) gives background sediment values for 5 lakes ranging from not detectable to 2.5 μg/g. Shepard (1976) discusses background levels of 0.3 - 6.2 μg/g.

The sediment cadmium concentrations resulting from the one year exposure to 5 and 10 μg/g water concentrations (Tables 6 and 7) were very low compared to values reported for contaminated field sites. Shephard (1976) found levels as high as 1300 μg/g in a lake contaminated by an electroplating plant and Kneip et al. (1974) reported levels of 3000 - 50,000 μg/g in an industrially contaminated sediment. The sediment Cd concentrations observed in our study were similar to those in the Derwent River, England (Harding and Whitton, 1978).

The low values observed in our work may be due to the low pH (Table 2) of the water. Murray and Mernke (1974) found virtually no Cd adsorption on suspended sediments at a pH of less than 6.6. The higher concentrations of Cd in the highly organic pool sediments is consistent with the finds of Riffaldi and Levi-Minzi (1975) who found that Cd adsorption maxima and coefficients were well correlated with cation exchange capacity and organic matter content. There is no immediately apparent reason why the tail pool sediments acquired higher Cd concentrations than the head pools although Korte et al. (1976) state that the percentageof clay in a sediment is the most useful predictor of whether or not a soil will retain a particular element. The tail pool sediments had a significantly greater clay content than the head pools (Table 1).

SECTION VII

AUFWUCHS

INTRODUCTION

As used in this report, the term "aufwuchs" refers to the complex epilithic, episalmic and epipelic assemblage of autotrophs and heterotrophs which developed on aquatic substrata. The German term aufwuchs was proposed by Ruttner (1953) to conote the community of both plants and animals attached to but not penetrating aquatic substrata. The term has been used interchangeably with the English term "periphyton" (Hynes, 1970); however, strictly speaking periphyton is only the autotrophic or plant component of the aufwuchs. We have separated the discussion of density and diversity of the micro and macro invertebrates from the general discussion of the aufwuchs community. These organisms, however, are included in estimates of standing biomass and metabolism. Qualitatively, the aufwuchs community includes algae, fungi, bacteria, protozoans, and small invertebrates and may form a mat up to a few centimeters in thickness depending on substratum orientation and current velocity. In flowing water systems, phytoplankton are virtually absent and the algae of the aufwuchs as well as macrophytic plants constitute the basis of the autocthonous food web. In well-lighted streams this in situ carbon production can be substantial and therefore the effect of Cd on aufwuchs dynamics is of considerable importance. The effect of Cd on the heterophic, non-algal components of the aufwuchs community are also of importance, since these organisms provide the mechanism for rapid cycling of nutrients and therefore sustained productivity in a lotic environment. The aufwuchs communities of aquatic ecosystems have been used as sensitive indicators of both chemical and physical stressors (Rodgers and Harvey, 1976) since it is sessile and taxonomically diverse and involved in all of the functional processes of ecosystems. For these reasons, the aufwuchs community is a biological integrator of ecosystem information (Weber, 1973). Wetzel (1975) states that the trophic structure above the producer - decomposer level, with all of its complexities, population fluctuations, metabolism and behavior, has relatively minor input on the carbon flux of ecosystems. The aufwuchs community is also the component with the greatest capacity to sorb potential toxicants.

In this study, three questions concerning the interaction of low Cd levels with aufwuchs were addressed: 1) What are the kinetics of Cd uptake and elimination and how are steady state concentrations in the aufwuchs related to water Cd concentrations? 2) What effect does Cd have on the structure of the aufwuchs community as measured by standing crop, species composition, pigment ratios, and chorophyll to biomass ratios? 3) How do any changes in the structure of the aufwuchs community affect system level functioning through primary productivity, metabolism, and export? In the scope of the above questions we are also asking if an aquatic system can adapt to a continuous toxin input and be dependent on it as an organizing influence.

METHODS

Community Structure

Two hundred 50 by 75 mm washed glass slides were placed in each channel on 1 November, 1975. These slides (referred to as long-term glass slides) were placed vertically on five notched racks holding twenty slides (Figure 2) placed 9 and 85 m from the head pools. These two groups are referred to as head samples and tail samples. Slides were held approximately 5 cm above the stream bottoms with their long axis parallel to stream walls. At monthly intervals four slides were randomly chosen from both the head and tail stations of each channel and placed in washed beakers. Both surfaces of the slides (area = 7500 mm^2) were carefully scraped and washed with a minimum volume of water (20 to 200 ml depending on aufwuchs density) and various determinations made as described below.

Beginning on 15 December 1975 and continuing at 8 week intervals, smaller sets of slides (6 to 9 at each position) were placed in the channels and allowed to colonize 30 days. Four slides were collected from each location and aufwuchs removed. These samples (referred to as short-term glass slides) were handled in the same manner as the long-term slides.

After scraping the slides a direct count of number of chironomids was made using a binocular dissecting scope at low power (30X). Densities of other invertebrates (copepods, caldocerans, ostracods, etc.) and large algae (*Eremosphaeria* and desmids) were qualitatively noted.

By November 1976, it was clear that glass slides were underestimating aufwuchs production on some substrata in the channels such as the walls, where the mat was several centimeters thick. This underestimation was due to some sloughing of aufwuchs as the slides were lifted from the water and also due to less accumulation on the slides because of their location in the central part of the streams where current was maximum. Because of these limitations samples were scraped directly from the stream walls and processed in the same manner as glass slide samples. Two samples (area = 587 mm^2) were collected from each wall in the head and tail portions of each channel on 8 November 1976, 12 January 1977, 30 March 1977, and 28 June 1977. These samples were then processed for algal cell densities by taxa, biomass, chlorophyll and Cd concentrations as described below.

On two occasions, six bottom samples (area - 426 mm^2) were collected from each channel to determine algal cell densities, biomass, chlorophyll, and Cd concentration. These samples were collected by inserting a piece of plexiglass tubing vertically into the sediment to the PVC liner. The entire core was placed in a clean flask and processed in a manner similar to glass slide or wall samples.

Of the diverse flora and fauna in aufwuchs samples, only the algal component was identified and enumerated. Identification to species was made when possible using standard taxonomic references, but in several cases common algae were not classifiable to species due to lack of fruiting stages or confusing taxonomic literature. Complete descriptions of all common species were made and there was little difficulty in distinguishing between them in routine counts.

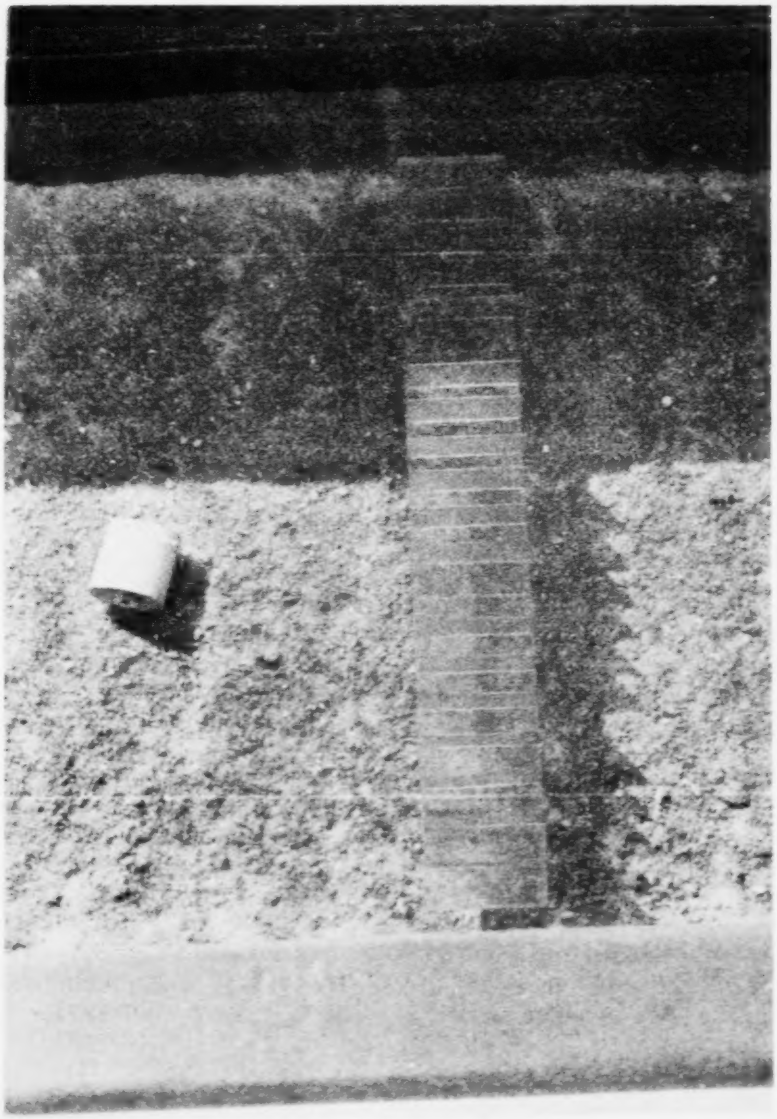


Figure 2. Glass slides used for sampling aufwuchs.

Scraped samples were blended for 30 seconds at low speed in a Waring blender to break up clumps and provide a more homogenous suspension for subsampling. Subsamples were taken by volumetric pipette for biomass, chlorophyll, and Cd concentration determination. A few milliliters of suspended sample was preserved with Lugol's iodine solution (APHA, 1976) for algal counts. Several aliquots of both living and preserved samples were examined and no significant differences found in cell densities or taxa observed. Also, samples were compared before and after blending and there was almost no qualitative or quantitative difference observed. However, one rare species (*Eremosphaeria viridis*) was known to be disrupted beyond recognition by either blending or preservation with Lugol's solution.

Algae were counted using the drop-transect method (Voelenweider, 1969). Samples were mixed, with a vortex mixer and 50 µl subsamples removed using an automatic microliter pipette placed on a clean glass slide and covered with a cover slip. Random transects were then counted using an ocular micrometer to delineate the width of the scanned field. All cells of living algae (intact cells with pigments) were counted as units and the number of cells per area of the original surface calculated using equation 1.

$$\text{cells/mm}^2 = \frac{484\ (N)\ (M)}{0.05\ (A)\ (T)} \qquad (1)$$

where: 484 = area of coverslip, in mm^2

N = number of cells counted

M = total sample volume, ml

A = area scanned, mm^2

0.05 = volume of 1 drop, ml

T = area sampled, mm^2

For interspecific comparisons cell densities were converted to volume of living cells. This calculation was made by measuring 50 cells of each species and estimating the average volume per cell using regular geometrical shapes including spheres, cylinders and ellipsoids.

Aufwuchs biomass was determined for a 10.0 ml aliquot from the mixed slurry described above. Aliquots were placed into pre-fired, pre-weighed crucibles, dried at 100° for 24 hr, cooled in a dessicator and reweighed. Biomass in grams per square meter of the original substrate (glass slide, wall or bottom) was calculated using equation 2.

$$\text{Biomass} = \frac{(M)\ (W)}{10\ (T)} \qquad (2)$$

where: M = total sample volume, ml

W = sample dry weight, g

T = sample area, m^2

Concentrations of chlorophylls a and b, carotenoids and phaeophytin pigment ratios were determined using the acetone extraction method of Strickland and Parsons (1972). Ten ml of mixed algal solution was filtered at 0.5 ATM through Gelman A-E glass fiber filters with a small amount of saturated $MgSO_4$ added as a buffer. The filter was ground in a glass tissue homogenizer, using a teflon pestle with several milliliters of 90% reagent grade acetone until it was completely disassociated. The total volume was adjusted to 10 ml with 90% acetone. The grinding tube was kept in a ice-water bath throughout the grinding period (approximately one minute). Blended samples were allowed to extract in the dark at 4°C for 24 hours. At that time the samples were centrifuged, decanted and re-centrifuged. Absorbance of clarified samples was measured at 750, 663, 645, 630, and 480 nm in a one centimeter cell using a Beckman ACTA-CIII Spectrophotometer. Extracts were acidified with one drop of 0.1 M HCl, and their absorbance remeasured at 750 and 663 nm. Absorbance values were corrected for turbidity with the 750 nm absorbance and background absorbance. Chlorophylls a and b and carotenoids were calculated according to Strickland and Parsons (1972). Acidification ratio was calculated by dividing the corrected 663 acidified absorbance into the 663 unacidified value.

Dried periphyton samples were wet ashed in 30 ml porcelain crucibles with 2 ml of concentrated HNO_3 at 80°C for 1 to 3 hours or until all solid material had dissolved and NO_2 evolution ceased. The samples were cooled, two ml of 30% H_2O_2 added, and reheated until gas evolution ceased. Samples were cooled to room temperature, diluted volumetrically using deionized water, and stored in washed polyethylene bottles. Analytical methods for cadmium are described in Appendix I.

Due to the heterogeneous assembly of organisms composing the aufwuchs community in the channels, an estimate of non-algal material was desired. Utilizing the estimation of algal volume and total biomass values suitably converted to live volumes, a percentage live algae was calculated for each sample. The live volume:biomass ratio was measured for a young growth of filamentous algae by lightly centrifuging cells from suspension, measuring their volume and determining dry weight. Dry weight (g) was 1.74 percent of live volume (cm^3). Percentage algal composition of the aufwuchs community was determined by dividing the volume of green algae (Chlorophyta) or blue-green algae (Cyanophyta) by total algal volume. Through the study these two groups together represented more than 95% of the algae present.

Preliminary comparisons of data have been limited to differences between treatments with replicate streams and head and tail locations pooled. At each sampling date the three values have been graphed and a test statistic calculated for comparison between any two of the means.

RESULTS AND DISCUSSION

Cd Accumulation

Cadmium levels reported for aufwuchs are on a dry weight basis for the entire community. At continuous Cd exposures of 0.05, 5.0 and 10.0 μg Cd/l, steady state concentrations in aufwuchs from long-term glass slides were approximately 3, 36, and 58 μg Cd/g dry weight (Fig. 3).

Concentration factors for Cd by aufwuchs were 7100X when exposed to 5 ppb and 5800X when exposed to 10 ppb which are similar to concentration factors reported by Gerhards and Weller (1977). Linear relationships between Cd concentration in culture media and algae have been reported by several workers (Gerhards and Weller, 1977; Payer et al., 1976; Kutagiri, 1975; and Kerfoot and Jacobs; 1976) however, the Cd concentration factor in the control channels was approximately 64,000 or 10 times the concentration factor for the higher Cd exposure concentrations. Other workers have found concentration factors of 500X for Chlorella pyrenoidosa (Hart and Cook, 1975); > 2000X for Analystis niclulans (Katagiri, 1975); 4000 - 6700X for marine diatoms (Kerfoot and Jacobs, 1976); 1000 - 2000X for bacteria and fungi (Doyle et al., 1975) 80000X for mixed algae (Kumada et al., 1973) and 10000X for marine phytoplankton (Knauer and Martin, 1973). Since Cd concentration factors are

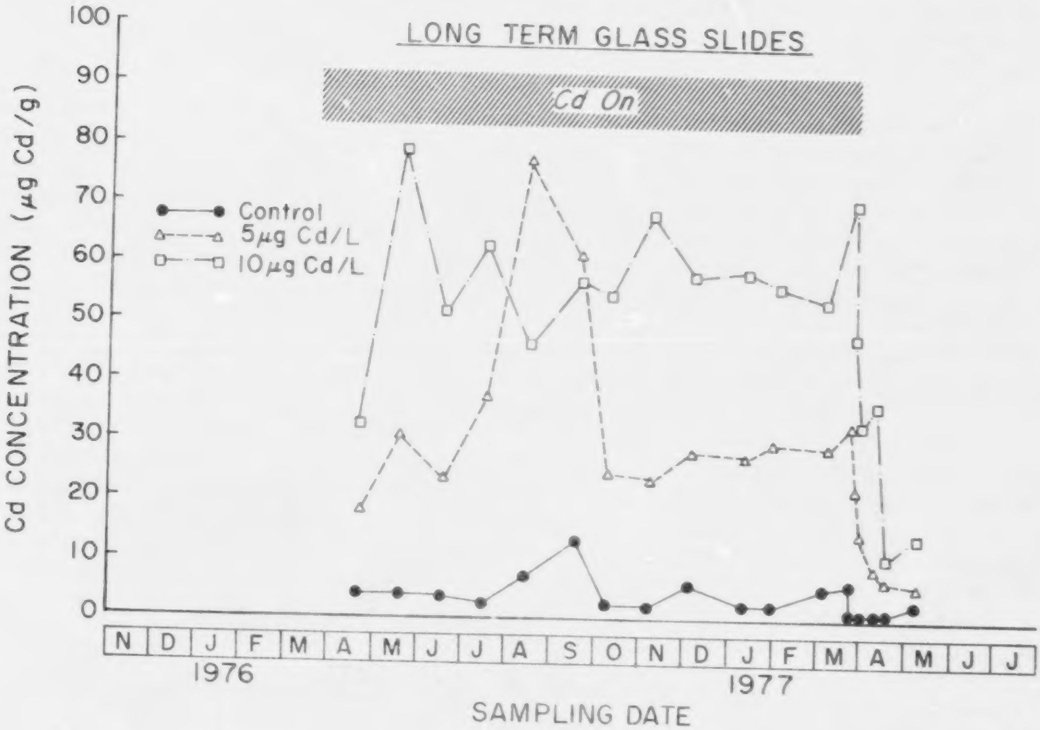


Figure 3. Mean Cd concentrations in aufwuchs collected from long term glass slides incubated in the channels from the beginning of Cd exposure.

related to available Cd in the medium and thus affected by the chemical and physical form which is determined by particulates, dissolved organics, water hardness (Kinkade and Erdman, 1975), orthophosphate levels (Motohashi and Tsuchida, 1974) and undoubtably to other chemical parameters, which affect the form in which Cd exists in water, it is not surprising to find a large range of factors reported for various algae and fungi.

Initial Cd accumulation was not measured but a charge up curve was observed with equilibrium reached within approximately 50 days (Figure 3). Katagiri (1975) and Kerfoot and Jacobs (1976) reported Cd accumulation by algae could be explained by a first order uptake model, which is consistent with our data.

The Cd concentrations reached in short term accrual experiments (23 days) (Figure 4) were essentially the same as the ambient steady state Cd concentrations observed on the long-term slides (Figure 3); indicating Cd uptake by the aufwuchs community was rapid. After Cd inputs ceased, water concentrations dropped to control levels within a few days. Cd concentrations on short-term glass slides incubated in the former treatment streams after input was stopped were not significantly different from controls.

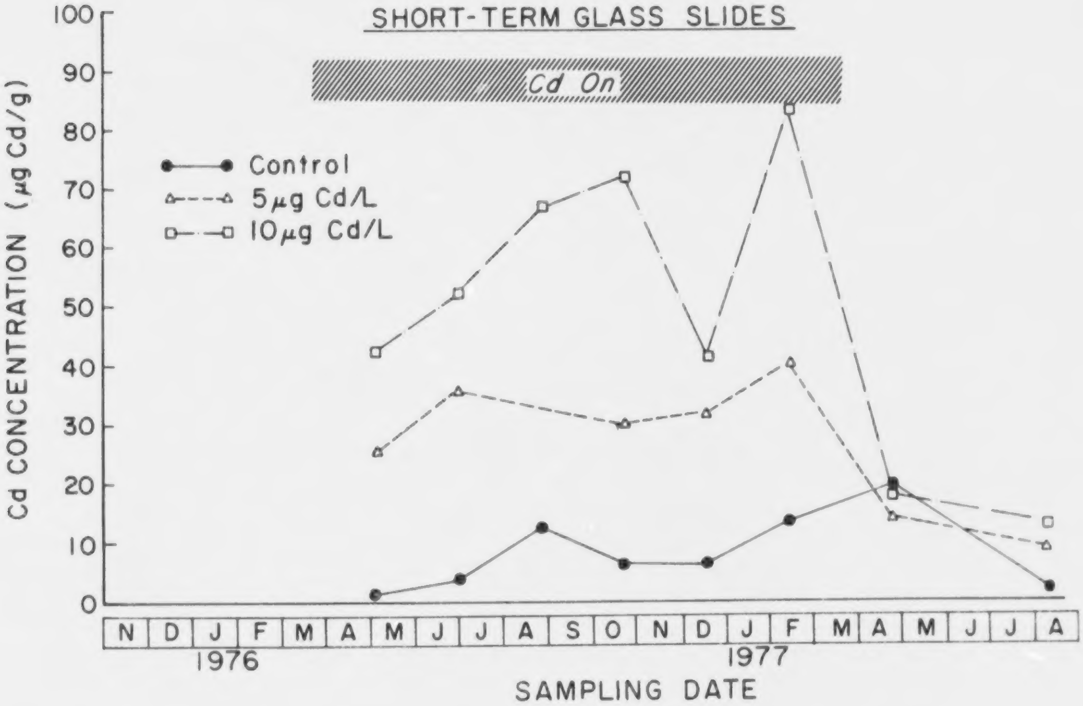
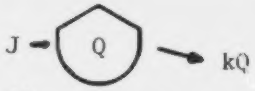


Figure 4. Mean Cd concentrations in aufwuchs collected from short term glass slides incubated in the channels for the eight weeks prior to sampling.

Cadmium uptake rate was calculated (Table 8) assuming a Von Bertalanfi growth model to describe Cd accumulation by aufwuchs (equations 3 - 6). Cadmium

TABLE 8. STEADY STATE CD CONCENTRATIONS IN AUFWUCHS AND UPTAKE AND ELIMINATION CONSTANTS

TREATMENT (μg Cl/ℓ)	STEADY STATE LEVEL (μg Cd g^{-1})	UPTAKE RATE (μg Cd g^{-1} day^{-1})	DECAY CONSTANT (day^{-1})
5	36	2.1	.06
10	58	3.9	.07



(3)

$$\frac{dQ}{dt} = J - kQ \qquad (4)$$

$$Q_t = \frac{J}{k}(1 - e^{-kt}) \qquad (5)$$

for steady state:

$$Q_{ss} = \frac{J}{k} \qquad J = Q_{ss}\,k \qquad (6)$$

where:

Q = Cd concentration in aufwuchs

Q_{ss} = steady state Cd concentration in aufwuchs

J = Cd uptake rate

K = Cd elimination rate

accumulation in the benthic aufwuchs was also determined on two occasions during Cd input using short term glass slides. The average values by treatment were: controls - 8, 5 μg Cd/ℓ - 75, and 10 μg Cd/ℓ - 116 μg Cd/g of ash-free dry weight. These values are approximately double the values found for the aufwuchs populations or vertical substrates and may be due to the relatively slower flushing of biological material from this storage.

Cadmium decay from the aufwuchs storage was followed in detail and data from the walls and glass slides is combined in a semi-log plot in Figure 5. The best linear fit for this data was found to be a single logarithmic decay for each treatment. Assuming a linear decay model, half-life values of 11.8 and 10.4 days were found for 5 and 10 μg Cd/ℓ channels respectively. Although the control aufwuchs appeared to lose cadmium during this period, the slope of the best-fit line is not significantly different from zero at the 95% confidence level.

Community Structure

After 20 months exposure to Cd, aufwuchs biomass was still increasing on long-term glass slides (Figure 6) and channel walls (Figure 7). Ultimate differences between standing crops on glass slides and walls indicate that glass slides underestimate standing crops on an areal basis at high aufwuchs densities. Before Cd input began, no appreciable differences were observed in aufwuchs standing crops between streams. Within two months after Cd input began, aufwuchs standing crops in channels receiving Cd were significantly lower than those in control channels (Figure 6). Standing crop values in the four treated channels remained similar to each other, but significantly lower than controls for five months at which time within treatment variance began to mask significant differences. After ten months of continuous Cd input, mean aufwuchs standing crops were similar across all treatments. Benthic aufwuchs samples measured after 11 months of Cd input had much greater biomass levels (controls - 157, 5 μg Cd/ℓ - 177, and 10 μg Cd/ℓ - 172 g ash-free dry weigh/ m^2) than the vertical substrates and also showed no significant differences between treatments.

Figures 8 and 9 summarize live algal volume on long-term glass slides and walls indicating that the apparent recovery observed for total aufwuchs biomass was not the result of algal recovery. Total algal volume declined in the treated channels shortly after Cd input began and remained at constant low levels throughout the rest of the study. Algal volume in the control channels was significantly greater than in the treated channels throughout the Cd input period, exhibiting a spring minimum and a late fall-early winter maximum. In bottom samples this trend was also observed with an average value of 22 cm^3/m^2 in the controls and 13 and 10 cm^3/m^2 seen in the 5 and 10 μg Cd/ℓ treatments, respectively.

Due to the similar effects of 5 and 10 μg Cd/l on algal volume, the required Cd concentration to depress community algal standing crop is less than 5 μg Cd/ℓ. Klass *et al*. (1974) reported 6 μg Cd/ℓ reduced *Scenedesmis gradricauda* growth and Katagiri (1975) observed growth inhibition of *Anacystis nidulans* at 50 μg Cd/ℓ. Conversely, Hart and Cook (1975) reported growth stimulation of natural phytoplankton populations by 11 to 110 μg Cd/ℓ. This may have been an indirect effect due to reduced grazing by zooplankton, which are very sensitive to Cd (Giesy *et al*., 1977; Marshall, 1977).

Aufwuchs standing crops showed little difference between treatments after 10 months of Cd exposure, however, algal population densities were significantly lower throughout the exposure period in channels receiving Cd.

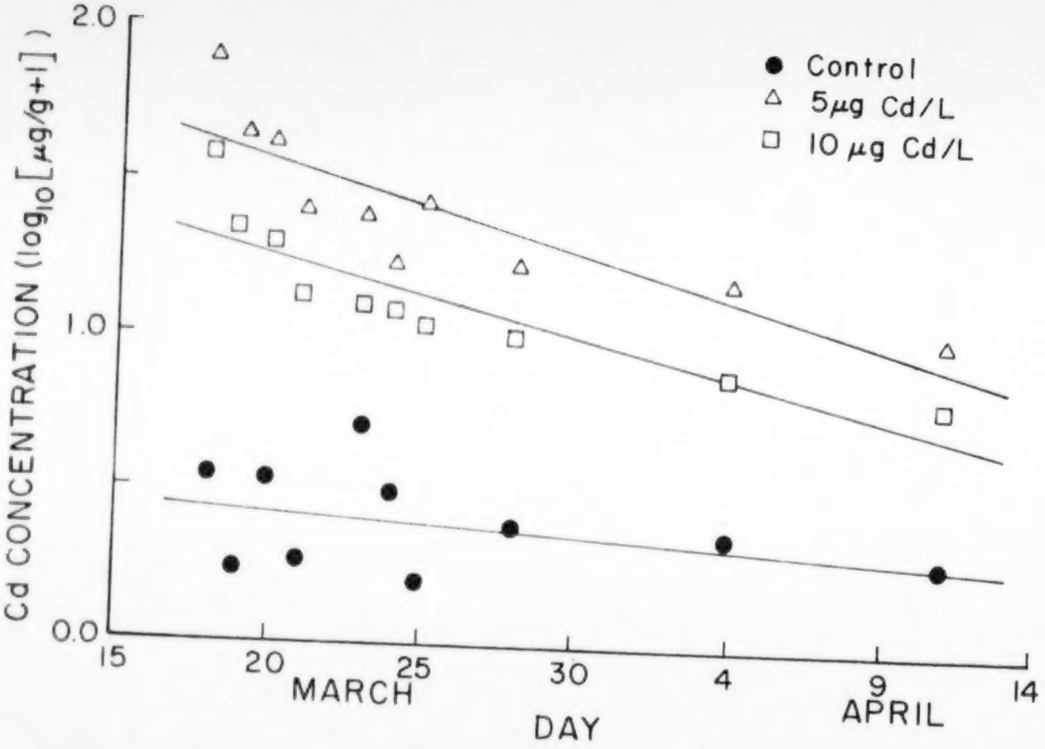


Figure 5. Linear regression of Cd elimination from the aufwuchs community colonizing glass slides.

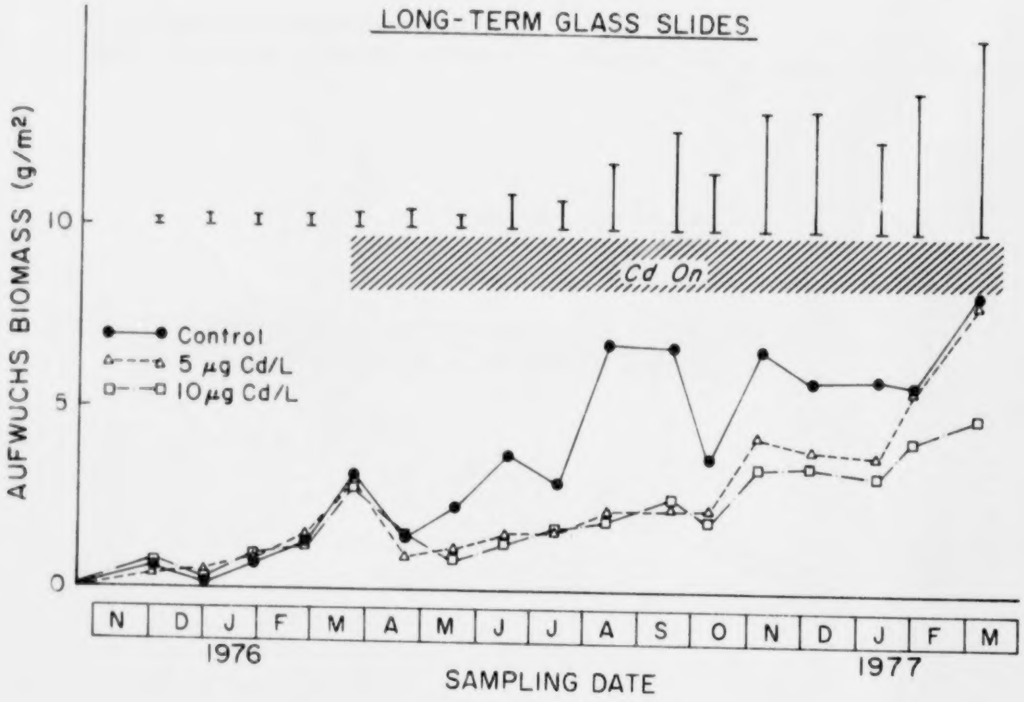


Figure 6. Mean aufwuchs biomass acrual on long term glass slides incubated from the beginning of the experiment with confidence intervals indicated.

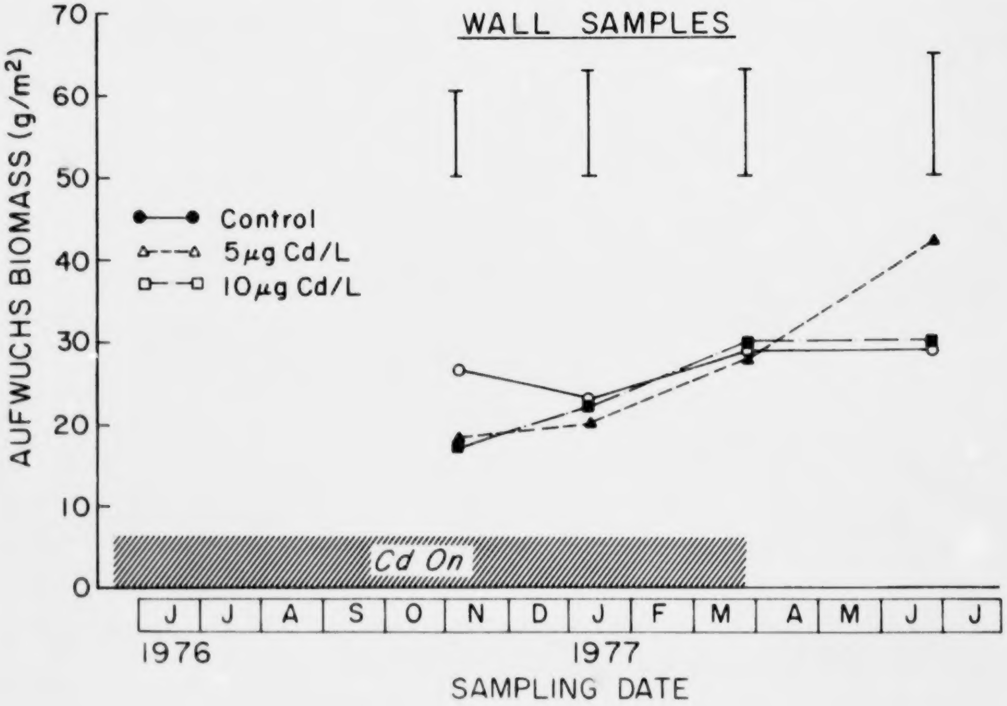


Figure 7. Mean aufwuchs biomass accrual on channel walls with two standard error confidence intervals indicated.

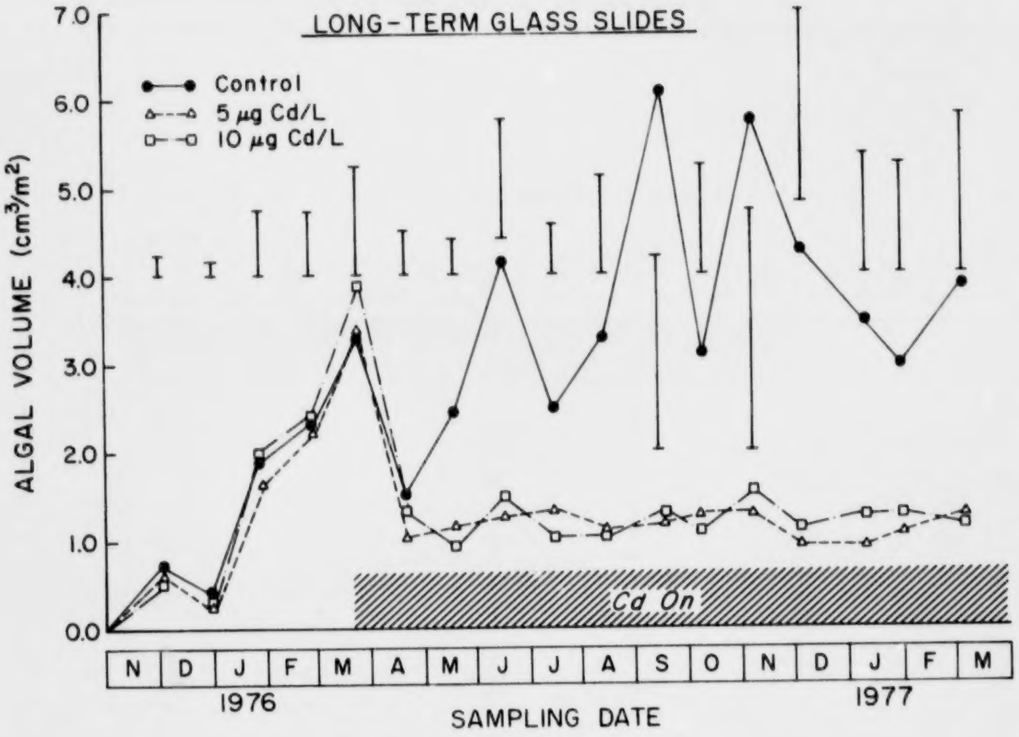


Figure 8. Mean viable algal cell volume collected from long term glass slides incubated from the beginning of the experiment with two standard error confidence intervals indicated.

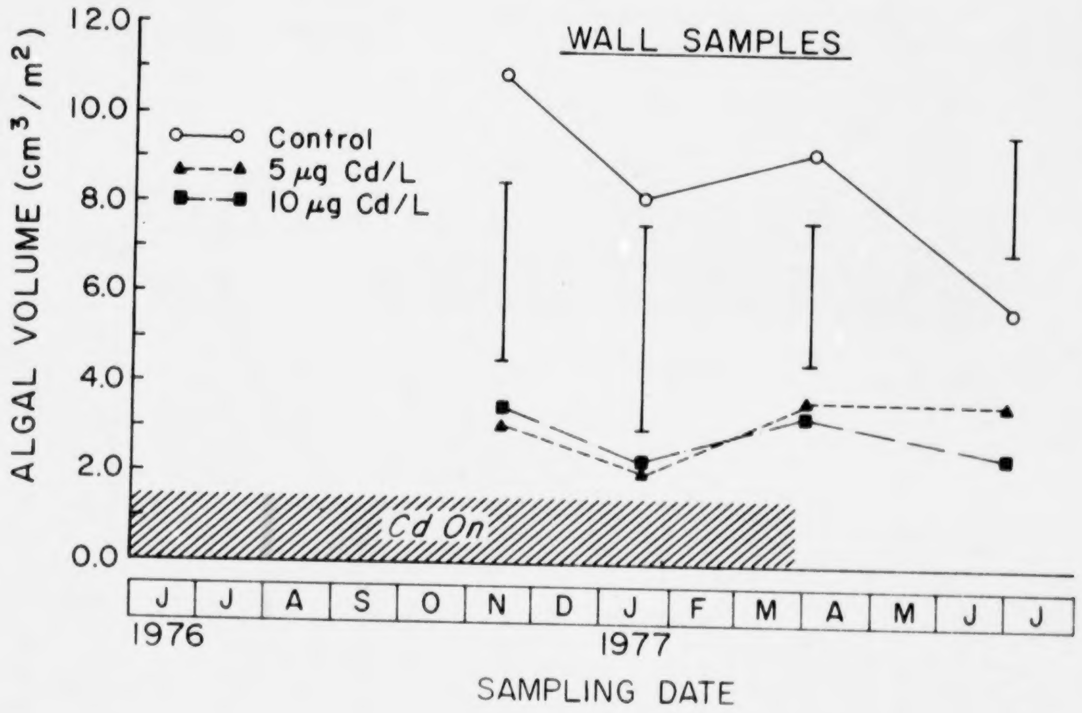


Figure 9. Mean viable algal cell volume collected from channel walls with two standard error confidence intervals indicated.

This decrease in the relative importance of the algal component in the aufwuchs community is reflected in a decreased chlorophyll a:aufwuchs biomass ratio (Fig. 10) which declined after initial colonization, due to accrual of photosynthetically inactive algae and heterotrophic organisms. This ratio was relatively consistent in all channels throughout the study. The chlorophyll: biomass ratio varied seasonally with significantly lower values during early summer and higher values during winter. Using an algal dry weight/ algal live volume X 100% value of 1.74%, determined for a relatively pure culture of stream algae, we found that throughout the study live algae made up 2% or less of the total aufwuchs dry weight.

Throughout most of the period of cadmium input, the aufwuchs communities were visably different in color. Communities in control channels were green to black while those in channels receiving Cd were orange-yellow. This observation was quantitatively verified by comparison of chlorophyll a to carotenoid pigments (Figure 11). In the control channels, a fall-winter maximum in the chlorophyll a:carotenoid ratio was observed corresponding to the period of highest algal standing crop. Communities in channels treated with Cd had significantly lower chlorophyll a:carotenoid ratios. Margalef (1961) and Odum and Hoskins, (1957) found that lower chlorophyll a:carotenoid ratios indicate a shortage of available nutrients. It is possible that nutrient recycling was limited by Cd and, therefore, algal populations were nutrient-starved. (See section XI). However, water nutrient levels were generally higher in channels receiving Cd than in control channels. An explanation for this paradox may be that most of the aufwuchs were actually exposed to much lower soluble nutrient concentrations under the surface layer of the mat and were dependent upon internal nutrient cycling.

Diatoms were rare in all samples from walls and glass slides regardless of treatment although several diatom species were observed in protozoan sponge samples. Nearly 100% of the algae were green (chlorophyta) or blue-green (Cyanophyta). Algal dominance shifted towards blue-greens with successional development (Figure 12). Samples from channel walls indicated a significantly higher percentage of blue-green algae in the Cd-treated channels.

Initial colonization in all channels was dominated by *Oscillatoria geminata* (filamentous blue-green), *Geminella turfosa* (filamentous green) and *Stigeoclonium elongatum* (filamentous green). These three species as well as several other filamentous greens and blue-greens, unicellular greens and blue-greens, and five desmid species were common in all channels throughout the study. A total list of algal species collected during the study is presented in Appendix (II).

Species diversity was calculated for the algal component of each aufwuchs sample using the formula derived from information theory (Pielou, 1969). Diversity ($\bar{H}$) initially decreased and then increased through the spring and summer to a plateau (Figure 13). The trend observed for diversity was largely due to changes in eveness of the algal population distribution as opposed to colonization by larger numbers of species (Figure 14). A few months after Cd input began, diversity values were significantly lowered in

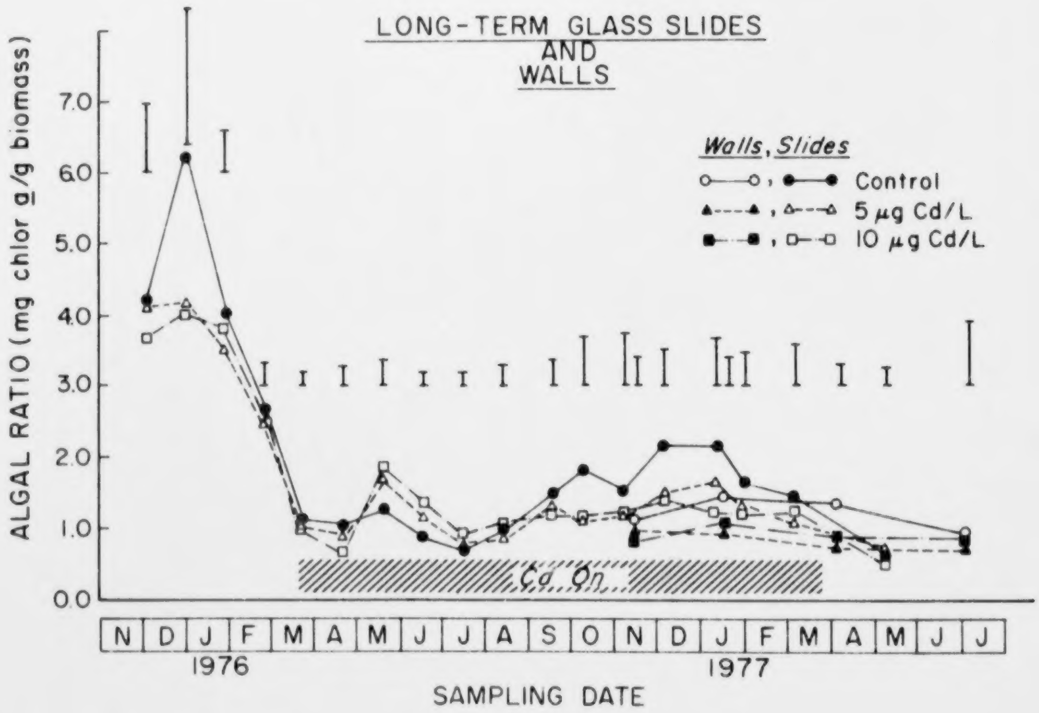


Figure 10. Algal ratio for aufwuchs collected from both long term glass slides and channel walls with two standard error confidence limits reported for long term glass slide samples.

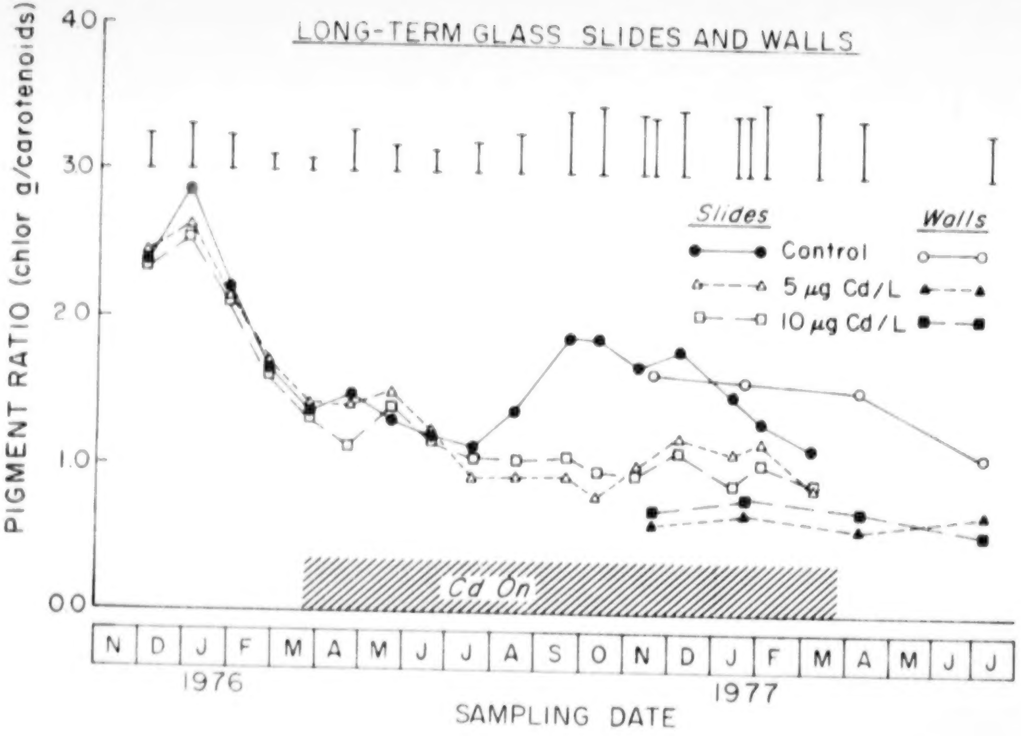


Figure 11. Pigment ratio for aufwuchs collected from both long term glass slides and channel walls with two standard errors confidence limits reported for glass wall samples.

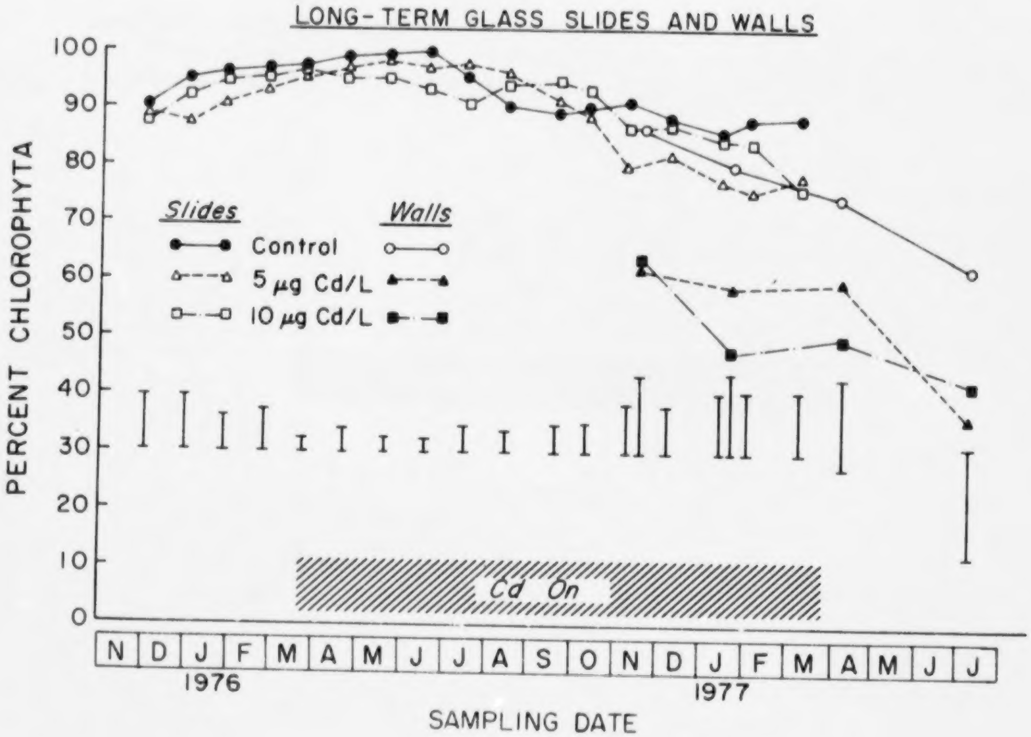


Figure 12. Percent of algal community, collected from long term glass slides and channel walls comprised of green algae with two standard errors confidence limits reported for long term glass samples.

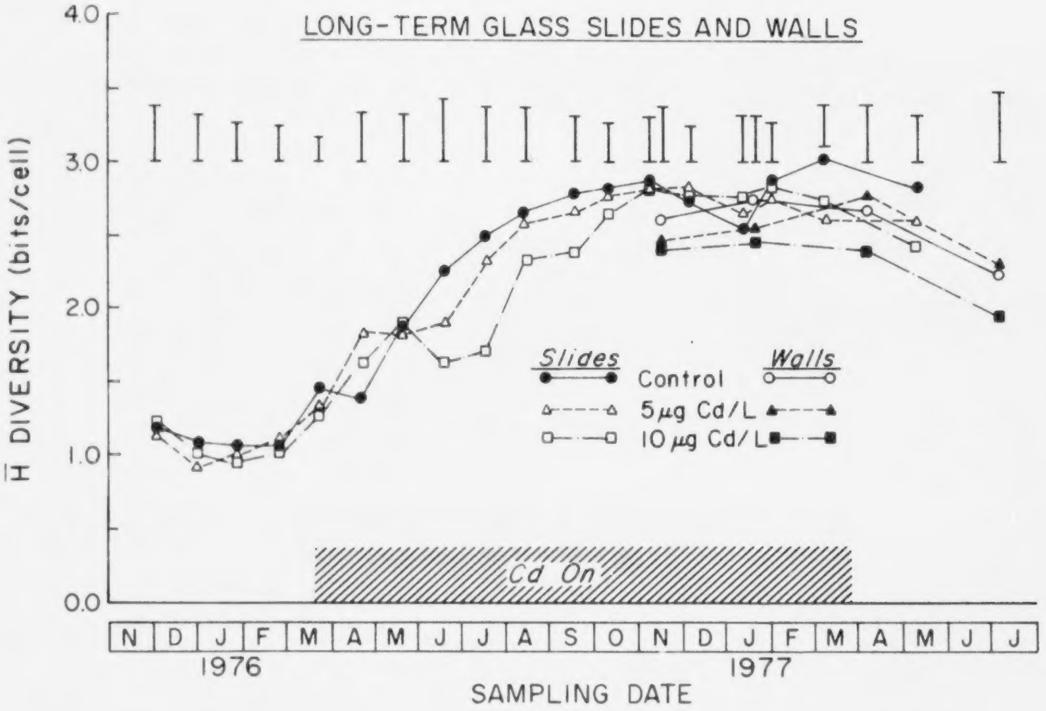


Figure 13. Diversity values for the algal community colonizing long term glass slides and channel walls with two standard errors confidence intervals indicated for long term glass slide samples.

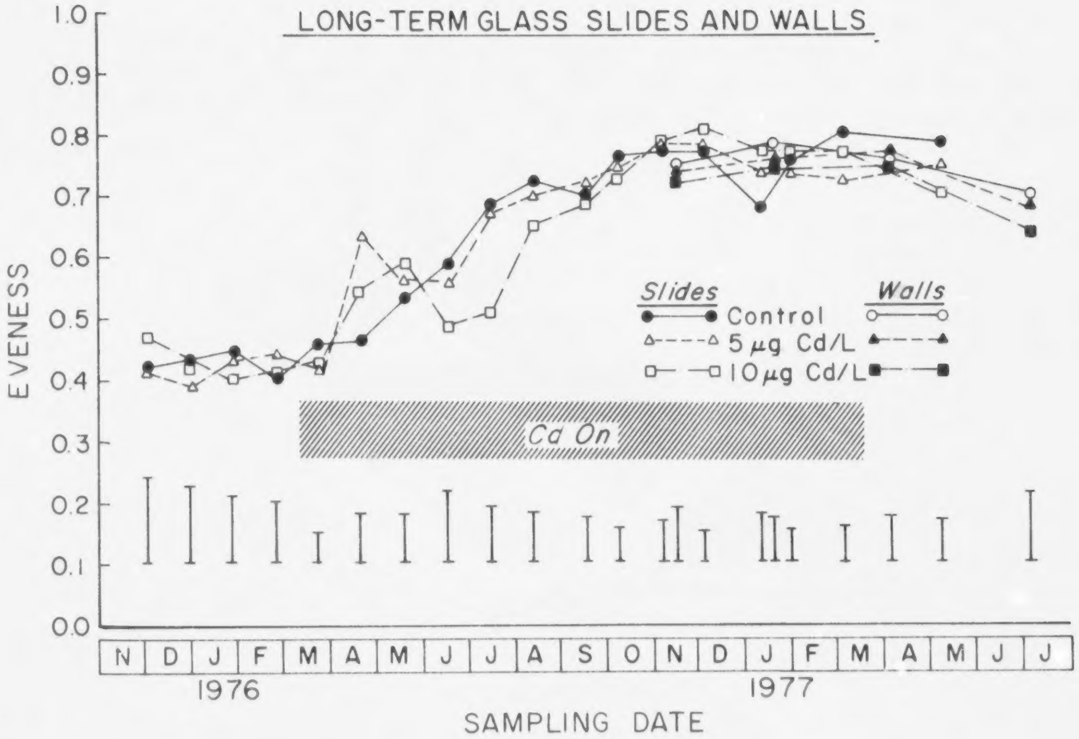


Figure 14. Evenness values for the algal community colonizing long term glass slides and channel walls with two standard errors confidence intervals indicated for long term glass slide samples.

the channels receiving 10 μg Cd/ℓ. Diversity reduction was less extreme at 5 μg Cd/ℓ and disappeared after seven months of Cd exposure.

Interactions

During the first 18 months of stream succession, aufwuchs provided the largest reduced carbon component for heterotrophic metabolism, and as indicated in Figures 6 and 7 was continuing to increase in standing stock at the end of the study. However, the total living algal contribution to the aufwuchs had apparently levelled off by the first year at which time the other autotrophic component of the streams (macrophytes) were rapidly increasing. It is possible that total algal volume in the streams would have continued to increase at a rate limited only by the increase in attachment surface area created by macrophytic plants.

All other organisms residing in the streams were by necessity dependent on algal and associated aufwuchs growth and would be expected to demonstrate indirect responses to Cd's effect on the aufwuchs component. Although the viable algal populations never showed recovery from Cd toxicity during the year after Cd input, aufwuchs levels recovered to comparable levels in all channels. The standing crops appear to have been largely limited by physical forces such as water current, torrential rains, and severe winds acting on them. In one respect we may now look at the densities of various consumers and try to understand how they were limited by algal populations (they should be lower in the treated channels) or by total aufwuchs biomass (initially lower in treated channels but gradually recovering during Cd input). However, we also have quite a different possibility that one might easily overlook. That is, the possibility that the consumers were directly affected by Cd treatment levels and a large part of the algal response was an indirect reaction to Cd, mitigated by decreased cycling capabilities of nutrients due to lowered ecosystem complexity.

We can examine these two possibilities and try to determine if one is more likely than the other based on the available data. The organisms found in the channels that would be dependent upon algae for nutrition and upon which the algae might be dependent for cycling of nutrients include: bacteria, fungi, protozoans, invertebrates and fish. For bacterial populations we have no pertinent information except for the specific group of nitrogen fixers. There was some indication that these organisms were adversely affected by Cd. Most likely this was a direct toxicity effect because of their ability to fix N_2 in the absence of an organic carbon source.

Fungi colonizing leaves in the tail pools were severely inhibited by Cd treatment (See Section XI). Since these organisms were given a allocthanous food source (dead tree leaves) their reaction to Cd would not be the result of a lack of energy, but rather a direct toxicity effect.

Microinvertebrate populations showed variable results with microcrustaceas and Difflugia reduced by Cd treatment and flagellates, cilliates and rotifers reaching higher population densities in Cd treated channels. Decreasing microcrustacean population densities in Cd treated channels may have been a result of reduced algal populations, since the single species of copepod and ostracod seen were vegetarian by nature. However the initial total disappearance of these organisms when Cd was added to the channels may more likely be the result of a direct toxicity effect which was mitigated by successional evolution in the streams, allowing their partial recovery with time. The increase in cilliate, flagellate, and roifer populations could not be the result of lower algal populations but may have contributed to those lower populations. Rather, as suggested elsewhere in this report, their increased populations may have been the result of tolerance to Cd toxicity as well as decreased predation and disease.

Macroinvertebrate populations also showed considerable differences between treatments and controls that may have affected algal populations. Mayflies and annelid worms were greatly decreased in the treatment channels and because of their food habits being largely non-algal, it may be assumed that their decreases were the result of direct Cd toxicity. The organisms which feed heavily on algae, e.g. the dipterans (Chironomidae and Ceratopogonidae) were stimulated to higher levels in the Cd streams in spite of lower algal populations. This stimulation of herbivores due to lack of competition and predators may have been largely responsible for the decreased algal populations observed.

Crayfish and Gambusia in Cd treated streams exhibited the greatest response to direct Cd toxicity. These populations were severely reduced compared to controls and indirect effects of these reductions were plainly seen. Reduced populations of crayfish in the treated head pools resulted in greatly increased macrophytic growth and a lowering of nutrient inputs to the treated streams while the loss of Gambusia from the treated streams no doubt played a big part in the increased herbivore populations.

So why then was the overall result of Cd input the reduction of algal standing crop and consequently primary production (and respiration)? Algal species capable of withstanding the direct toxicity effects of Cd were in the treated streams as evidenced by luxuriant growths at isolated times and locations. What were the effects of Cd that indirectly inhibited algae and primary production? A likely explanation in need of further investigation is the effect of Cd on the higher organisms, the heterotrophs or consumers, and with the disruption of their balanced web of relationships by an exogenous poison and the subsequent lowering of sustainable autocthonous energy fixation. Several studies with widely different organisms have shown that primary production may be maximum at levels of consumers greater than zero but less than maximum possible populations (Cooper, 1973; Hargrave 1970; Flintard and Goldman, 1975). Normally a system will be forced to adjust population densities of the various consumers in a series of feedback steps, each leading to a slightly greater fixation of utilizable energy. In the time scale of our study this natural system selection could be carried only so far because the ultimate consumer (Cd) was being maintained at a fixed level without feedback control. Natural systems, receiving a toxin such as Cd, might indeed over a long enough time derive mechanisms controlling the toxin's concentration. Possible examples of mechanisms already found for Cd are sequestering of the element in non-living materials such as humics and losses through sedimentation. Natural systems may be adapted to use environmental levels of Cd as a controller, or consumer in their tuned networks resulting in maximum sustainable energy utilization. Thus the difficult question of what attributes of a system should be protected (see Section XIV).

SECTION VIII

MACROPHYTES

INTRODUCTION

In shallow standing or sluggishly moving water, emergent macrophytes are often a dominant feature of aquatic communities. These macrophytes exert a strong influence on the community in several ways. Their most obvious contribution is in the production of fixed carbon which is available to the heterotrophic system components. These plants may also exert a strong controlling influence by their utilization of available nutrients and light and the release of soluble organic compounds which then affect the algal components of the ecosystem. Also important is the physical habitat the macrophytes provide for invertebrates and small vertebrates. With respect to the relative importance of macrophytes, Westlake (1975) states that the role of macrophytes in the aquatic community lies more in their role in modifying and diversifying habitats than in the supply of organic matter.

Aquatic macrophytes are able to concentrate Cd from both water and sediments (Harding and Whitton, 1978) and may serve as a source of Cd to herbivore populations.

Macrophyte populations were monitored to determine the effects of Cd on colonization and growth as well as Cd accumulation by macrophytes.

METHODS

Clumps of *Juncus diffusissimus* plants growing in a pond adjacent to the artificial streams facility were transplanted into the channels and tail pools on March 15, 1976. Thirty clumps spaced at 2 m intervals were put into the channels and 5 clumps were put into the tail pools, which at the time contained crayfish. The plants introduced into the tail pools were quickly eliminated by the crayfish except in one case (tail pool 6) where there was 100% mortality of the crayfish. This supports the hypothesis that the early natural colonizing macrophytes in the channels were eliminated by the crayfish. The number of live shoots and height of the tallest shoot in each clump placed in the channels was monitored monthly until October 1976. "Live" shoots were considered to be those shoots extending to or upwards from the water line and containing chlorophyll. Emergent and submergent shoot samples were taken monthly from three clumps for Cd analysis. No attempt was made to take root samples because of the damage this would have done to the plants.

By June of 1976, sufficient numbers of large J. diffusissimus had developed in the head pools for destructive sampling of these populations to be initiated. Starting at that time, a single plant was removed monthly from each pool, divided into shoot and root portions, dried, and subsamples taken for digestion and subsequent Cd analysis. In September 1976, a similar sampling program was initiated for J. diffusissimus naturally colonizing the channels. Individual plants were removed from both ends of each channel and prepared for Cd analysis. Callitriche heterophylla became relatively common in the channels and pools by November 1976, and a monthly sampling program for this species was begun at this time, again both upstream and downstream stations in each channel were sampled.

By the time Cd input was terminated (March 1977), population densities of naturally colonizing macrophytes were high enough so that plant biomass sampling by quadrat analysis was feasible. A survey was made in conjunction with a large scale invertebrate sampling program. Ten 0.25 m^2 sections of sediment and associated plants were removed from each channel. A logarithmic sampling distribution was used so that most samples were taken from the upstream reaches of the channels, which were most heavily colonized. All plants were washed, sorted by species, counted, dried in a forced air oven at 100°C, and weighed to the nearest 0.1 g. From these data, macrophyte biomass per unit area was calculated. The entire sampling procedure was repeated using different quadrats in September 1977 in order to ascertain any changes in macrophyte biomass that occurred after six months of recovery.

All samples taken for Cd analysis were rinsed free of sediment and periphyton, and placed in plastic bags in which small holes had been punched. After the samples were freeze dried to constant weight, subsamples of 0.05 -0.10 g were refluxed in previously fired (900°C, 1 hr.) porcelain crucibles with 2 ml redistilled concentrated HNO_3 at 85°C on a hotplate until evolution of NO_2 ceased. Samples were cooled to room temperature, treated with 1 ml 30% H_2O_2, heated until clear, cooled, and diluted with repeated deionized water washings of the crucibles.

RESULTS AND DISCUSSION

In a previous study with the channel microcosms (Kania et al., 1976), the emergent macrophyte J. diffusissimus became an increasingly important component of the channel communities as time progressed, especially in the upstream reaches. The growth of this rush at the heads of the control channels was so extensive after two and one-half years that the water flows became restricted and the systems had to be channelized. Because of the seeding technique used for this study, the same Juncus was expected to again become a dominant community member and the macrophyte analytical program was designed with this in mind.

Shortly after the initial seeding of the channels in October 1975, two types of macrophyte seedlings were observed in the channels. These plants persisted only until December, however, and then disappeared. The disappearance may have been caused by the feeding activity of the crayfish introduced into the channels in December or the onset of cold weather, or the plants may simply not have been suited to the environment of the channels. By the end of January 1976, the channels and tail pools were completely devoid of rooted macrophytes of any kind although the head pools contained a relatively dense growth of young macrophytes (Table 9). The persistence of macrophytes in the head pools which had no crayfish indicates that crayfish were responsible for the elimination of macrophytes in the channels and tail pools.

Data obtained during the first three months of sampling from the transplanted *Juncus diffusissimus* clumps indicated that the plants in the treated channels may have been losing shoots faster than those in the control channels.

TABLE 9. DISTRIBUTION AND NUMBER OF INDIVIDUAL MACROPHYTES IN HEAD POOLS AS OF JANUARY 31, 1976

Head Pool	*Juncus diffusissimus*	*Gratiola virginiana*	*Callitriche heterophylla*	*Bacopa caroliniana*
1	227	26	1	0
2	499	18	1	0
3	439	19	0	0
4	396	8	1	0
5	576	15	2	1
6	429	22	1	2

This phenomenon disappeared, however, during the following four month period, during which all clumps produced new shoots in about equal numbers. No differences in macrophyte growth rate were noted between treatment or control channels at any time. All measurements on the transplanted clumps were discontinued after October 1976 because clumps were too large and overgrown with algae to be effectively counted and measured.

By September 1977 when *Juncus* naturally colonizing the channels was sampled for Cd, there were major differences between treated and untreated systems with respect to macrophyte populations (Figure 15). Control channels had many more plants than channels receiving Cd inputs. However, there were no apparent differences between the 5 μ g/ℓ and 10 μ g/ℓ treatments. These observations were confirmed by the macrophyte biomass sampling carried out in March 1977 (Figure 16). Figure 16 also shows the distribution of the macrophytes in the channels with the greater population densities existing near the input wiers. This same pattern was observed in a previous study (Kania *et al*., 1976).

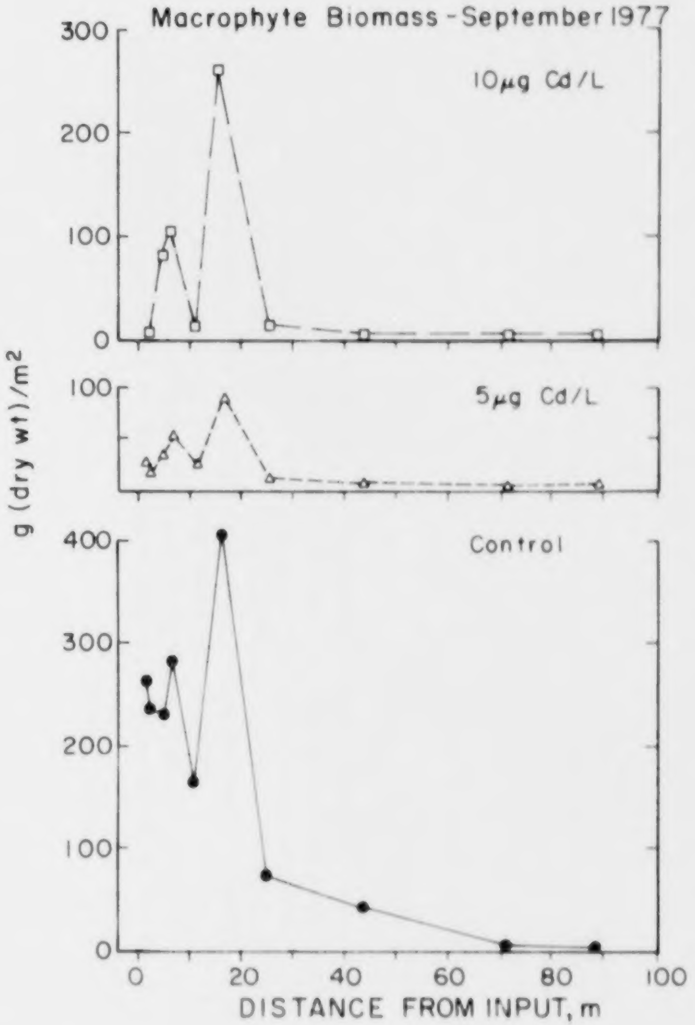


Figure 15. Macrophyte standing crop biomass as a function of distance from the head-pools, as of September 1977.

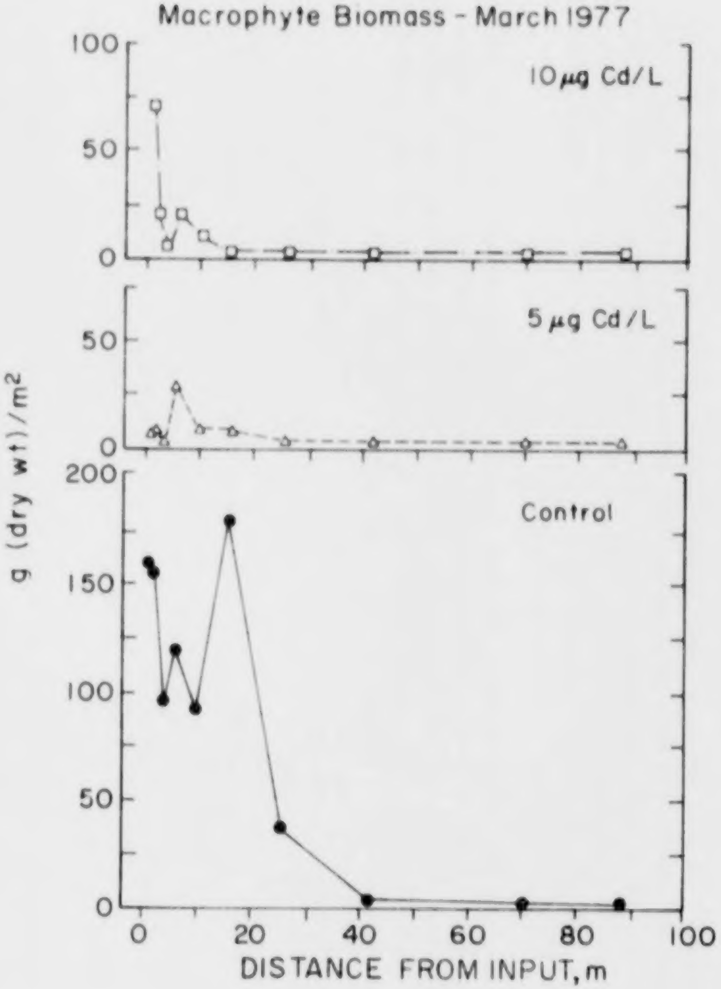


Figure 16. Macrophyte standing crop biomass as a function of distance from the head-pools, as of March 1977

The biomass in all systems approximately doubled in the six month period with no cadmium inputs (Figure 15), with some trend toward recovery indicated in at least the 10 μ g/ℓ treatments (Figure 16). The macrophyte distribution in the channels remained unchanged. The colonization pattern observed was probably due to turbulence near the head and distance from seed source and nutrient input.

Fleischer et al. (1974) state in their comprehensive review of Cd in the environment that "reports of cadmium toxicity symptoms in plants grown under field conditions have not been found." This is probably because the necessary long term studies that include several species have not previously been done. Because of the possibility of food chain transfer of Cd to humans a number of laboratory uptake and plant toxicity studies have been done with agricultural plants (John et al., 1972; Page et al., 1972; Francis and Rush, 1973; Haghiri, 1973; Turner, 1973; John et al., 1976; Petterson, 1976; Koeppe, 1977; Reddy and Patrick, 1977; Wallace et al., 1977) including forage species (Bingham et al., 1976). These studies, which have in general been carried out with extremely high levels of Cd, have demonstrated that the most common response of plants to Cd is reduced growth (i.e. yield) although chlorosis has also been reported, and that the levels of exposure required to elicite a toxic response varies tremendously not only with species but even with variety (John et al., 1976).

The amount of information on Cd effects on aquatic plant species is limited. There have been two recent studies (which have included some investigation of effects) although they were primarily concerned with Cd uptake by Spartina (Dunstan and Windom, 1975; Dunstan et al., 1975). These studies showed that germination of Spartina seeds was not affected by Cd exposure concentrations of up to 100 μg/mℓ. In growth studies over an eight week period, this concentration had no effect on growth rate or net primary production. It is not clear as to whether the sensitivity of J. diffusissimus to Cd as demonstrated in our study was simply a species difference or related to the softwater medium in which the Cd was presented. Cearley and Coleman (1973) working with the fresh water naiad Najas quadulepensis found that plants exposed to Cd levels as low as 7 μg/ℓ demonstrated reductions of chlorophyll, turgor, and stolen development, although they do not relate the extent of these responses to the doses used.

CADMIUM ACCUMULATION

The cadmium concentrations measured in macrophytes from the control channels and head pools (Table 10) were similar to those reported by other workers for freshwater macrophytes from uncontaminated areas (Cearley and Coleman, 1973; Gommes and Muntau, 1976; Lee et al., 1976) but are generally higher than those reported for a marine form (Dunstan and Windom, 1975; Dunstan et al., 1975). The two species studied here appear to be very similar in their ability to concentrate Cd from dilute solutions (Figure 17, 18, 19, 20 and 21. In both species, roots were in all cases higher than the leaves, (Figure 17, 18, 20 and 21) an observation made by others for aquatic plants (Lee et al., 1976; Gommes and Muntau, 1976).

TABLE 10. CADMIUM CONCENTRATIONS IN MACROPHYTES REMOVED FROM CHANNELS NOT RECEIVING CD.

Sample	N	$\bar{x}$ ug/g	SD	CV %
Callitriche heterophylla				
Natural colonizers				
Leaves	10	1.05	1.94	185
Roots	28	8.55	6.96	81
Juncus diffusissimus				
Natural colonizers				
(channels)				
Leaves	54	1.48	0.96	65
Roots	55	6.19	3.51	56
(Head pools)				
Leaves	32	0.70	0.92	131
Roots	31	2.43	1.44	59
Transplants				
Leaves				
(Emergent)	94	0.61	0.84	138
(Submergent)	94	0.75	0.98	130

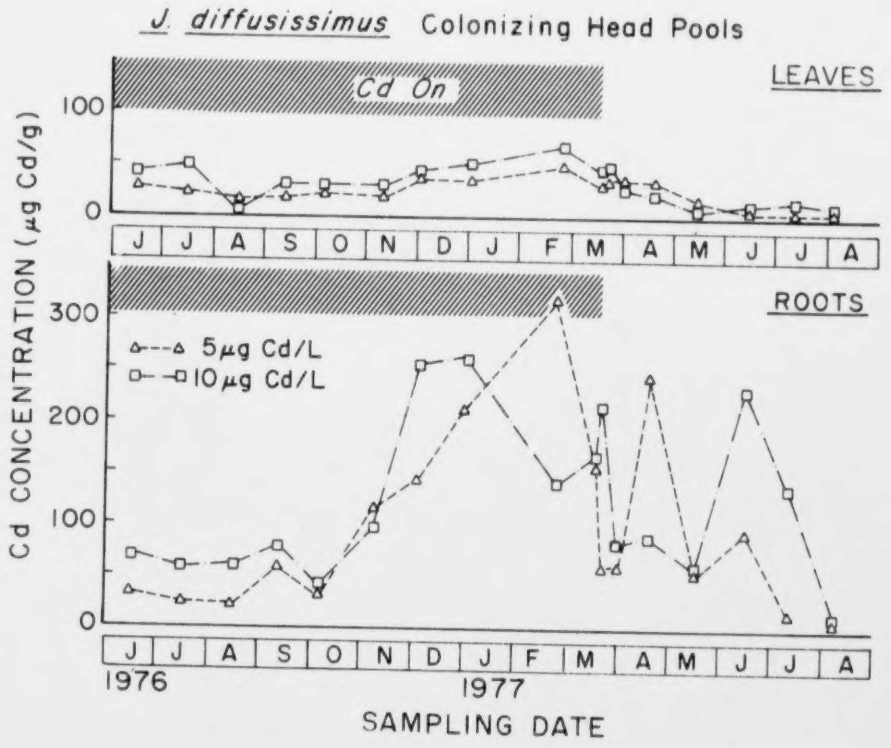


Figure 17. Cadmium concentrations in J. diffusissimus colonizing the headpools, expressed on a dry weight basis.

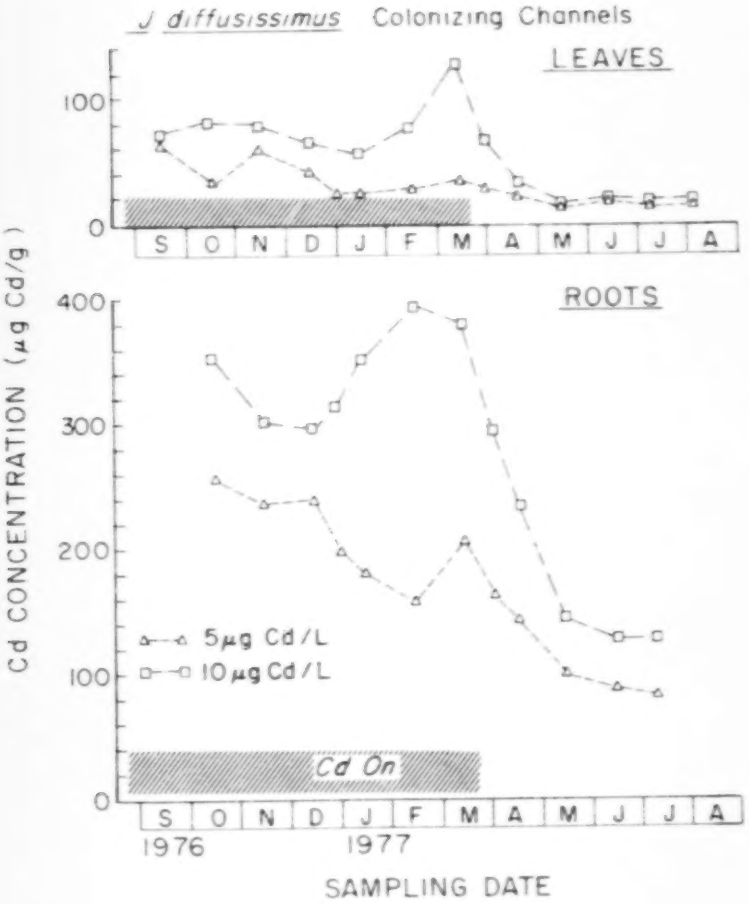


Figure 18. Cadmium concentrations in J. diffusissimus colonizing the channels, expressed on a dry weight basis.

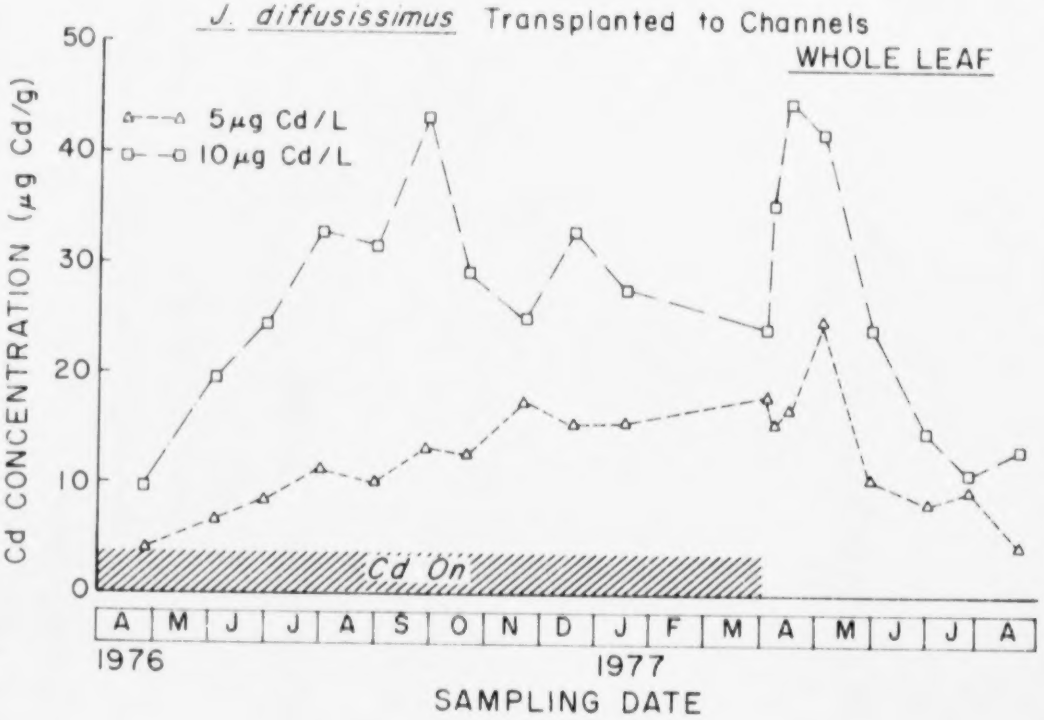


Figure 19. Cadmium concentrations in J. diffusissimus transplanted to the channels, expressed on a dry weight basis.

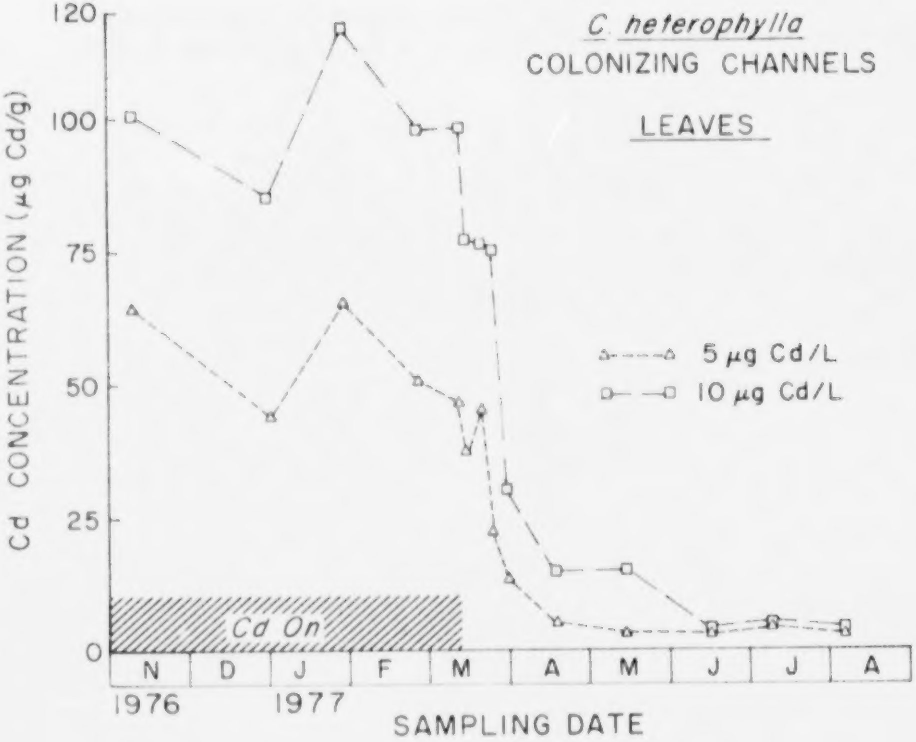


Figure 20. Cadmium concentrations in C. heterophylla shoots colonizing the channels, expressed on a dry weight basis.

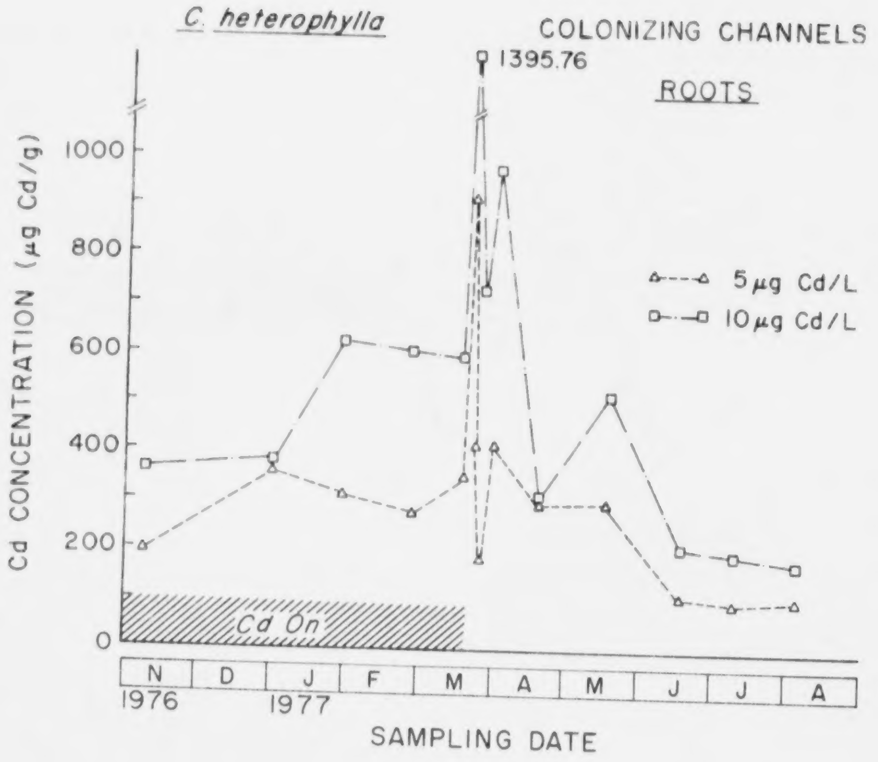


Figure 21. Cadmium concentrations in C. heterophylla roots colonizing channels, expressed on a dry weight basis.

J. diffusissimus plants transplanted into the channels were lower in Cd throughout the study than the naturally colonizing forms that developed there. The sediment in the pond from which the transplanted *J. diffusissimus* were removed was much higher in organic matter content and clay than the channel sediments. *J. diffusissimus* that colonized the highly organic natural sediments in the head pools had lower Cd concentrations than those in the channels and quite similar to the transplanted plants. In the transplanted *J. diffusissimus* there were no differences between submergent and emergent portions of leaves with respect to Cd concentrations, and the concentrations observed are not to any extent due to loosely adsorbed Cd or Cd associated with unremoved periphyton.

No difference was observed between emergent and submergent portions of leaves of transplanted *J. diffusissimus* so these have been averaged (Figure 19). In Cd treated systems, the naturally colonizing *Callitriche heterophylla* and *J. diffusissimus* leaves and roots were at equilibrium at the time sampling was initiated (Figure 17, 18, 20 and 21). This is not surprising considering the rapid rate at which at least some aquatic plants can take up this metal (Wolverton, 1975; Cearley and Coleman, 1973). We did not observe the great species differences cited by other workers (Petterson, 1976; Fleischer *et al*., 1974), and in the channels, both species concentrated Cd to a similar degree although the roots of *C. heterophylla* were generally contained greater Cd concentrations than did those of *J. diffusissimus*. This may be due to the finer roots of *C. heterophylla* and thus greater potential uptake surface.

In all samples roots contained greater Cd concentrations than did leaves. Also, naturally colonizing *Juncus* in the head pools (Figure 17) accumulated less Cd, than those growing in the channels (Figure 18). Cadmium uptake patterns were different in these two regions in several ways; (1) there was little difference in Cd uptakes by *J. diffusissimus* growing in the pools exposed to 5 μg Cd/ℓ or 10 μg Cd/ℓ. Conversely, Cd uptake by *J. diffusissimus* growing in the channels was approximately proportional to that which they were exposed, (2) there was a general increase in Cd levels in *J. diffusissimus* roots growing in the headpools during exposure to Cd, while there was a decrease in root Cd concentrations in plants growing in the channels, (3) Cd concentrations in roots, especially those made after Cd inputs were terminated, were much more variable in samples from the head pools than in those from the channels.

During the five month period after Cd inputs were stopped, Cd concentrations in plants growing in both the head pools and channels declined to similar levels, even though sediment Cd levels in head pools remained constant and greater than channel sediments. These results are consistent with those of John *et al*. (1972) which demonstrated that plants grow on soils with increased Cd adsorbing capacity and increased organic matter content had lower Cd concentrations than those grown on soils with lower Cd sorbing capacity (see Table 1).

Transplanted *J. diffusissimus* (Figure 19) never reached Cd levels as great as those acquired by the naturally colonizing plants. Wolverton (1975) working with water hyacinths stated that mineral uptake rates per unit of dry matter are greater for plants in a rapid growth phase, and decrease as the plant ages. J. diffusissimus clumps transplanted into the channels in March 1976 contained at least one season's growth and after a small growth period in the spring, did not measurably increase in size. Cadmiun concentrations in the leaves of transplanted J. diffusissimus increased rapidly during the month after Cd inputs were terminated. There is no apparent explanation for this observation.

In all cases, Cd concentrations reported here for macrophytes from Cd treated systems are high relative to other researchers. Lee et al. (1976) reported root levels of 61 and 18 μg/g dry weight for Scirpus and Cyperus roots growing in medium containing 500 μg/ℓ Cd and leaf levels of 3-20 and 20-65 μg/g for nonrooted portions of these same species. Cearley and Coleman (1976) report Cd levels of 60 μg Cd/g ash for Najas exposed to 7 μg Cd/ℓ. Our high results may be the result of the extremely soft acid water of our system.

SECTION IX

INVERTEBRATES

INTRODUCTION

The importance of macroinvertebrates as essential components in aquatic systems is well documented (Hynes, 1957; 1960; 1970; Weber, 1973; Cummins, 1973; Brinkhurst, 1974; Cummins, 1975; and Carins, 1977). Carins (1977) states that, aquatic macroinvertebrates are important components in food webs of aquatic systems, being primary and secondary consumers, and serving as food sources for higher trophic levels. While Cummins (1973) states, the role of macroinvertebrates in the overall structure and function of stream and river ecosystems is the conversion of reduced carbon compounds derived primarily from the surrounding land supplemented by in-stream carbon fixation, into temporary storage in their own tissue and into carbon dioxide.

Numerous surveys have used the aquatic communities of streams, rivers, and lakes as indicators of water quality. The works of Gaufin and Tarzwell (1952 and 1956) and others have demonstrated that the composition and distribution of benthic invertebrate communities are useful tools in evaluating perturbations in aquatic systems due to various types of pollutants.

Macroinvertebrates are especially well suited for such studies because: 1) their limited mobility does not allow for perturbation avoidance, 2) their ubiquitous distribution in aquatic habitats, 3) the relative ease by which they are collected and in many cases identified, 4) their fairly long life cycles, which means that once a perturbation has affected a community's composition and/or distribution, it generally requires an extended period of time before new recruitment can reestablish the original community structure. Cairns (1977) and Cairns et al. (1972) have suggested macroinvertebrates (predominantly protozoans) are good indicators of environmental stressors.

Recently, much effort has been spent studying the acute toxicity, uptake, and accumulation of heavy metals in aquatic organisms (Ravera et al., 1973; Thorp and Lake, 1974; Karbe et al., 1975; Club et al., 1975^{a}; 1975^{b}; Nehring, 1976; and Enk and Mathis, 1977). However, only limited information is presently available on the use of aquatic invertebrates in assessing the effects of potentially harmful chronic levels of heavy metals in laboratory as well as in natural systems.

The objective of this portion of our study was to determine the fates and effects of chronic Cd exposures (5 and 10 μg/ℓ) on benthic organisms under controlled, semi-natural conditions. To accomplish these goals, steady state levels and uptake and elimination rates of Cd were determined for a number of taxa. Partitioning and Cd dynamics due to growth and molting cycles of organisms were also studied. Finally community measures were employed to assess effects of Cd on the structure and function of the macrovertebrate community.

METHODS AND MATERIALS

Macroinvertebrates

Cadmium effects on the benthic community as well as individual benthic populations were assessed by identification and enumeration of individuals collected using artificial substratum samplers and random bottom samples. The major portion of the macroinvertebrate survey relied heavily on multiple plate artificial substratum samplers. Multiple-plate samplers were selected because: (1) Multiple-plate samplers provide a practical means of collecting macroinvertebrates in a system, where destructive bottom sampling would devastate the system. (2) Plate samplers permitted quantitative comparison between treatments as well as rapid and consistent sample handling. (3) Multiple-plate samples, although they may exclude some taxa, do collect a sufficient diversity of benthic forms to be useful in relating benthic populations to watr quality (Fullner, 1971).

Samplers utilized were modified from those described by Hester and Dendy (1962), Fullner (1971) and EPA (1973). Samplers were constructed of 3.2 mm double-tempered "masonite" cut into 7.6 cm square plates and 2.5 cm square spacers. Each sampler consisted of 13 plates and 31 spacers. The "masonite" plates and spacers were positioned on stainless steel rods, resulting in three each of single spaced, double spaced, triple spaced, and quadruple spaced plates. Each sampler had an effective sampling surface of 0.16 square meters. Four stainless steel support racks, each supporting three samplers were suspended at each sampling station (Figure 22). This arrangement allowed sampling after both short-term and long-term exposure periods. The short-term incubations were of six weeks duration (APHA, 1975; Weber, 1973). One rack with three replicate samplers was removed from each sampling station at six week intervals and replaced by a new set of samplers. Samplers were preserved in 75% ethanol for subsequent sorting and enumeration. The long-term sampling program required three sample racks of three replicate samplers each at every sampling station. One sampler was removed every 12 weeks over an eighteen month period and preserved for enumeration. This procedure allowed us to collect samplers which had been exposed in the channels from the first day of the macroinvertebrate program (September 1975), until the end of the project.

Plate sampler removal was accomplished by enclosing each sampler in chambers constructed of plexiglass and stainless steel screen (mesh size 0.589 mm) prior to removal, to minimize the loss of organisms from samplers (Figure 23). Detritus and organisms collected were scraped from the samplers and preserved in 75% ethanol. Sample volumes were reduced by filtering them through #15 silk bottling cloth (mesh size 0.095 mm) before sorting.

Samples were also collected by removing 0.25 m^2 of bottom sediment enclosed by two stainless steel screens (mesh size 0.589 mm) pressed into the substratum. The bottom material removed was diluted with tap water and the suspended material passed through a U. S. standard #30 sieve. Detritus and associated organisms retained by the sieve were placed into a white enamel pan, the living organisms were removed with forceps and preserved with 75% ethanol. This procedure was repeated until the sand substratum produced no

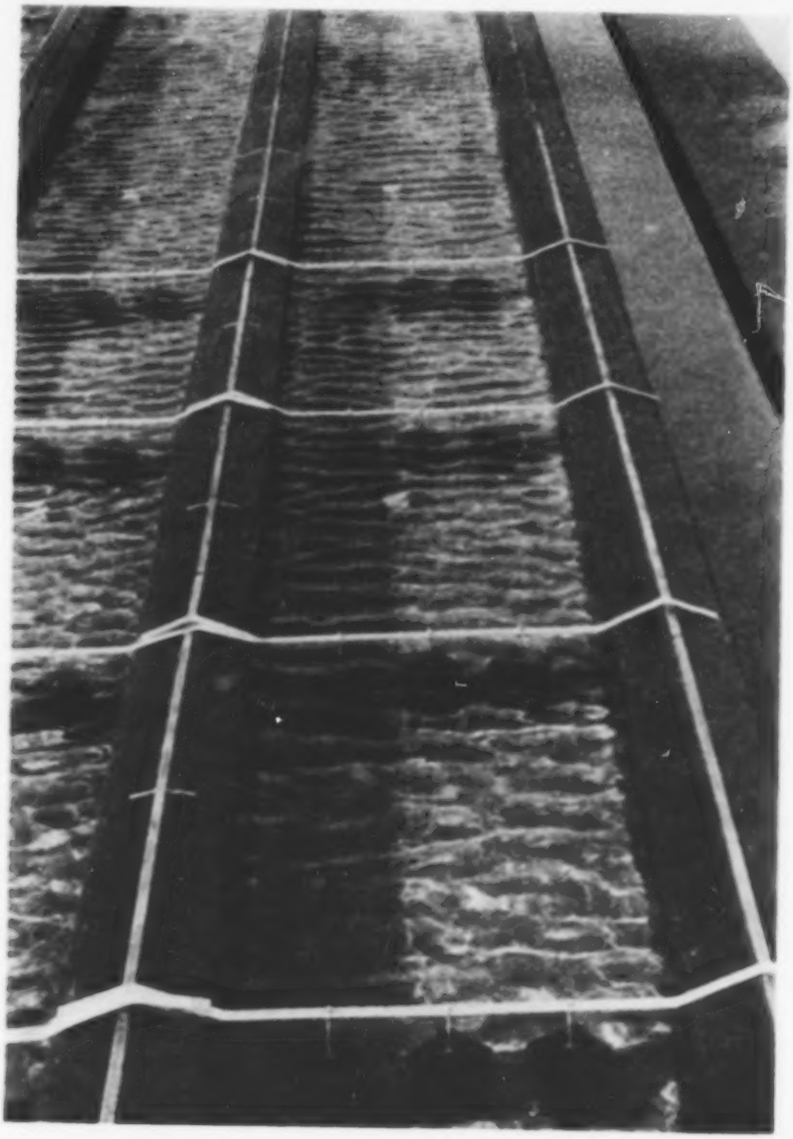


Figure 22. Hester-Dendy type invertebrate samples suspended in channels.

additional organisms. The remaining sand substratum, amalgamate and detrital material was returned to the location from which it was removed.

Macroinvertebrates were sorted microscopically into taxa regardless of size or instar. All representatives of each taxon were placed in labeled vials and stored for future identification and enumeration. Samples were sorted twice to assure complete collection.

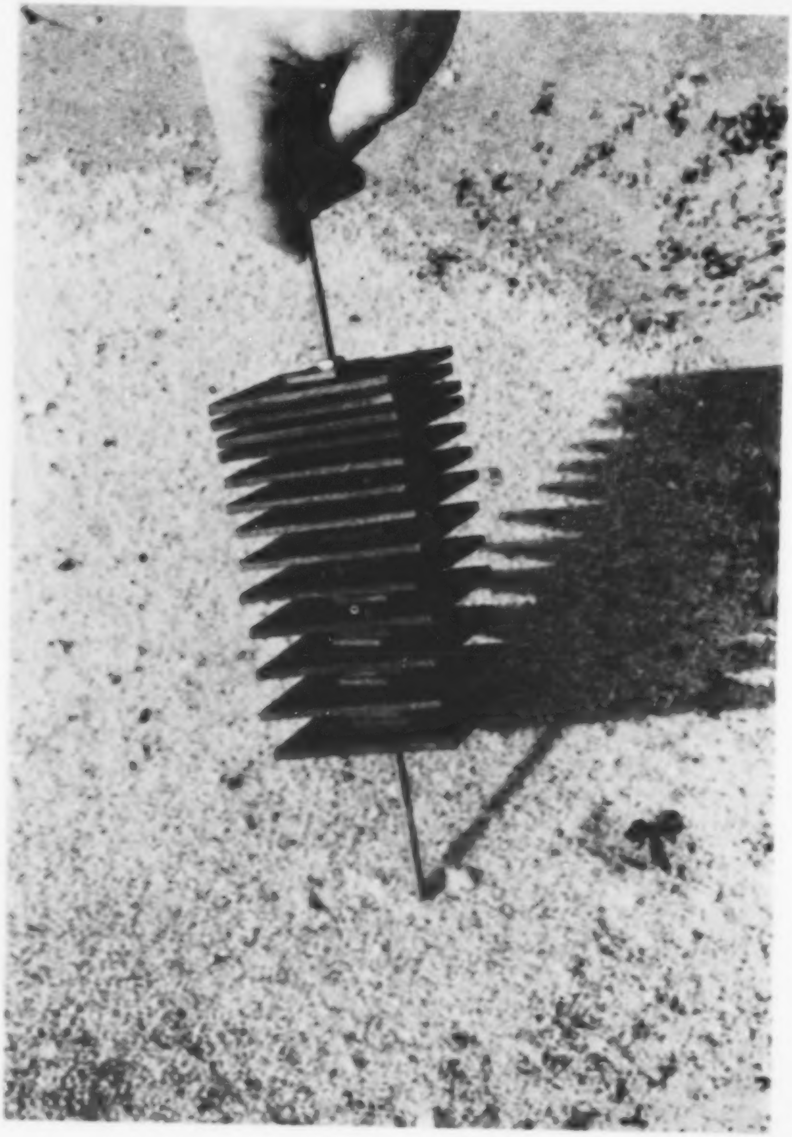


Figure 23. Invertebrate sampler in plexiglass and screen sampling box.

The sieving and sorting of macroinvertebrate samples was somewhat different than procedures described in *Standard Methods* (APHA, 1975) or in *Biological Field and Laboratory Methods* (Weber, 1973). In these procedures, as in most classical works, macroinvertebrates were defined as those invertebrates retained by a U. S. standard #30 sieve (0.595 mm mesh opening), while all other invertebrates were considered microinvertebrates. These artificially created categories, as observed by Jonasson (1955), Mundie (1971), and Mason *et al*. (1975), result in the selective retention of certain species and, in general, the elimination of smaller instars (developmental states) and/or taxa. Because of the limited literature available on long-term chronic effects of trace elements on benthic communities, it was unclear as to what role early developmental stages might play in overall community structure and function. Therefore, we have redefined macroinvertebrates to include not only those organisms retained on a U. S. sieve #30, but all recoverable instars of those organisms, regardless of size. Also considered when making this decision was the potential for increase in sample size. Johasson (1955) found that by utilizing small mesh sieves he could achieve a 100 - 600% increase in numbers of individuals captured over collections made using a sieve of 0.6 mm opening. He also observed that the 0.6 mm mesh sieve was inefficient in collecting small Chironomid larvae. This result is crucial to our study where the Chironomid larvae may comprise 75 - 100% of the benthic population, depending on season and stage of channel colonization.

Macroinvertebrates collected in this study were generally identified to genus and occasionally to species, the Chironomidae being the only exception. The assemblages of Chironomidae collected by our techniques contained numerous small instars. Therefore, due to the difficulties in taxonomy of closely related groups and especially among younger instars, identifications were made from a limited number of samples throughout the study.

Monthly insect samples were collected randomly from natural substratum samples for all treatments. Because of the seasonal scarcity of particular taxa and the prohibitive amount of time required to collect equal sample sizes of each taxa, sample collection guidelines were necessary. The decision was made to collect individuals of all available taxa within the time interval required to collect 25 Chironomids. As a result, in all taxa except Chironomidae, the number of individuals comprising a sample may vary both within and between sampling periods for any treatment.

The organisms necessary to comprise a sample were taken randomly from available substrata in each treatment, using a small nylon screen net (mesh size 0.589). Samples were placed in plastic containers prior to sorting. Organisms were sorted from small aliquots of substrata placed in enamel trays and removed using stainless steel forceps. Upon removal, organisms were rinsed with deionized water and placed into clean plastic vials containing deionized water. Each taxon was placed in a separate vial and stored frozen until digestion.

Immediately after emergence and prior to flight, adult dragonflies and their corresponding exuvium were collected by hand, wherever possible, placed in plastic bags, and stored frozen prior to preparation. This collecting method allowed for the direct comparison of adult and exuvium Cd concentrations in individual dragonflies.

Insect samples except those of the larger dragonflies (adults and nymphs) were digested as follows: samples were partially thawed, poured into a fired porcelain crucible and completely thawed using deionized water. Thawed organisms were then removed from the crucible using stainless steel forceps and placed on acid washed, dried and tared platinum dishes. Platinum dishes were then placed in small petri dishes and dried at 50°C in a drying oven to obtain a constant dry weight. Dried samples were weighed on a Cahn Model 2500 Electrobalance. All platinum dishes and samples were then placed into acid washed 1 ml glass volumetric flasks for digestion. The taxa and number of individuals per sample were recorded. Except for pooled samples of Chironomids and Ceratopogonids, all other samples contained individual organisms. Insect sample digestion was accomplished using 60 µl of redistilled conc. HNO_3 and 20 µl of 30% H_2O_2. Volumetric flasks containing samples were heated in a water bath at 60° to 70°C (this procedure did not adversely affect the platinum dishes). Digestion was determined to be complete by the formation of a clear pale yellow solution in each flask. After complete digestion, samples were allowed to cool and platinum dishes were removed with an acid washed glass hook and rinsed with several drops of deionized water over the flask. Sample volumes were adjusted to 1 ml with deionized water and samples were ready for analysis.

Samples of larger insects, dragonfly adults, nymphs and exuvia were freeze-dried to obtain constant dry weight and weighed. Samples were digested in fired porcelain crucibles using redistilled conc. HNO_3 (0.6 ml for adults and nymphs; 0.4 ml for exuvia) and 30% H_2O_2 (0.2 ml for adults and nymphs and none for exuvia) at 60° - 70°C on a hotplate. After complete digestion, samples were rinsed into 5 ml glass volumetric flasks and brought to volume using deionized water.

Monthly *Corbicula fluminea* samples were collected from each treatment, by removing four transplanted organisms from the tail region of each channel. The entire soft body of these organisms was dissected out of the shell using a stainless steel surgical sealput, placed in a plastic bag and frozen prior to digestion. *C. fluminea* samples were analyzed using both flame and flameless atomization techniques. Flame atomization was required for clams exposed to 5 and 10 µg Cd/l and flameless methods were used for control and background organisms. Matrix interferences were encountered with flameless atomization techniques and tissue Cd concentrations were determined using standard addition techniques (see Appendix I).

C. fluminea samples were freeze-dried to obtain constant dry weight. Samples were digested in fired porcelain crucibles using 1 and 2 ml of redistilled conc. HNO_3 and 0.5 or 1 ml of 30% H_2O_2 depending on tissue size (large or small) on a hotplate at 50°C for 10 to 15 hr. Samples were diluted to 25 ml with deionized water after cooling.

Macroinvertebrates

The usual benthic sampling methods are biased toward collecting larger invertebrate forms in aquatic systems. Even by using a smaller screen for sorting purposes, a number of organisms relatively important in aquatic food chains cannot be collected. We attempted to consider, at least in a qualitative manner, Cd effects on these smaller invertebrate forms by utilizing an aritificial substratum sampling method developed by Cairns (Cairns et al., 1969, Cairns and Ruthren, 1970). This method uses polyurethane sponges suspended in the water as a habitat for colonization which can be sampled with replication and a minimum disruption of the study area. This method has been successfully used for studying the colonization and succession of freshwater protozoans (Youngue and Cairns, 1971) and also the response of protozoan communities to chlorine stress (Cairns and Plafkin, 1975).

The sponges provided an ideal sampling substratum for a wide variety of organisms in addition to protozoa. Indeed, the sponges could have been used to sample the algae component of the eocsystem. Several algal species were found only in samples from the sponges.

Rinsed polyurethane sponge cubes, 5 cm on a side (Figure 24) were suspended at two locations in the channels and repeatedly squeezed to exclude all air. Two new pairs of sponges were placed at each station monthly and sampled two weeks later. Only one pair of sponges was examined, the other provided a back-up capability in case of sample loss.

Sponges were squeezed dry by hand into a 500 ml beaker and immediately mixed. Two-2 ml samples were taken and placed in counting chambers. The total remaining volume of water was measured and a 100 ml aliquot preserved with 5 ml of formalin. Samples were allowed to stand for approximately one hour in the settling chamber. The total chamber volume was then examined at a magnification of 56 X with a Wild M 40 inverted microscope and the total number of larger forms identified and enumerated. This magnification allowed the enumeration of the larger protozoans, rotifers, nematods, anelids, flatworms, insect larvae, ostracods, copepods, cladocerans and occasionally, taridgrads, mites, and gastrotrichs. After the larger forms were enumerated, the samples were examined at 560 X. Ten random fields were completely counted. Although some minute flagellated forms did not "settle" in the chamber, these constituted a relatively small portion of the total microinvertebrate population. No attempt was made to derive a species identification for all of the multitude of forms observed because of the time constraints imposed by the use of living materials, and the lack of taxonomic expertise; however, consistently observed forms were identified.

Youngue and Cairns (1971) demonstrated that water contained in sponge samplers may differ from the surrounding medium at least with respect to pH. To determine if organisms inhabiting sponges suspended in the treated systems were actually exposed to Cd, a series of sponges was submerged in Cd spiked water for a period of two weeks. The Cd concentration of water squeezed from the sponge was not significantly different from that in the surrounding medium.

Counts made in the sponge material were pooled into larger taxonomic groups because of the small numbers of each species. Each time period was analyzed separately by ANOVA techniques for treatment effects. There was no significant ($P \leq 0.05$) difference between upstream and downstream stations. So data from these stations were pooled by treatment.

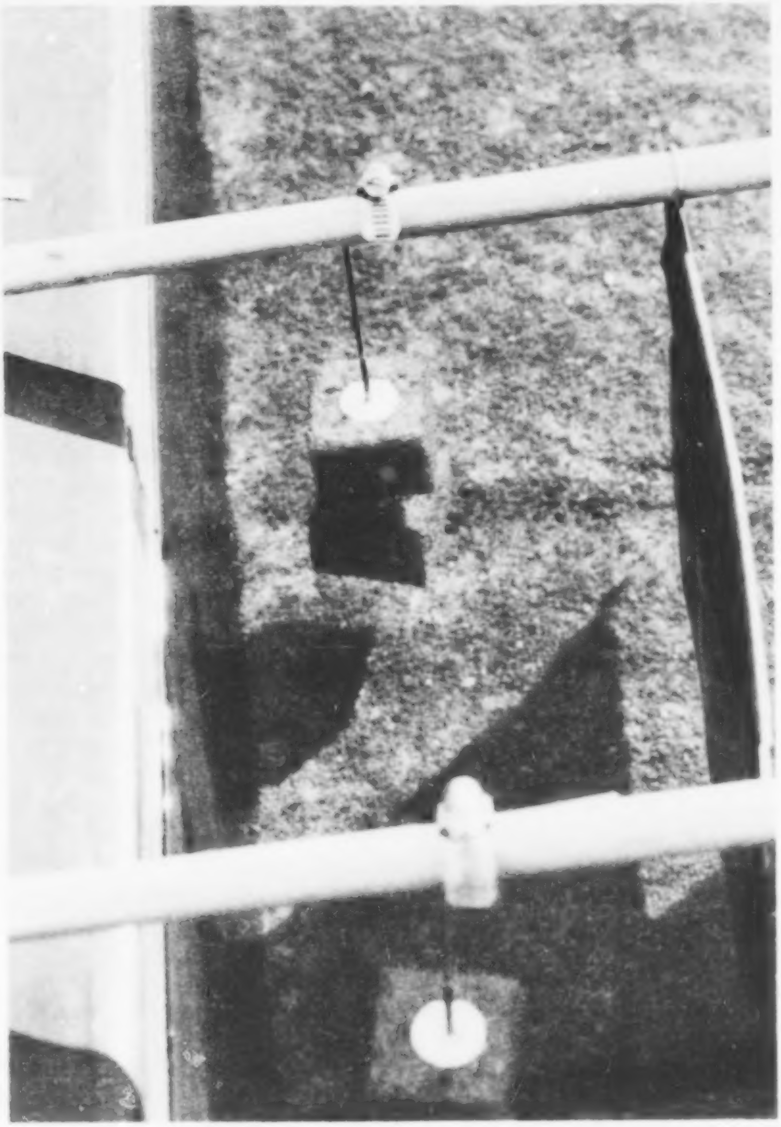


Figure 24. Polyurethane sponge microinvertebrate samplers.

RESULTS AND DISCUSSION

Cd Accumulation

Information on Cd accumulation and elimination by aquatic macroinvertebrates (ordinarily insects) is incomplete in this study due to the complexities and confounding properties of the natural environment. Factors which were determined as being necessary to appropriately calculate uptake and elimination rates along with concentration factors included: 1) the potential for multiple routes of exposure, uptake and elimination; 2) seasonal effects on population level (emergence and recruitment), development cycle (alterations in metabolism, size, shape and molting rate), feeding habits such as selectivity and habitat selection; and 3) alterations in environmental availability and exposure levels of the metal in question. All of these reasons combined to make it quite apparent that in order to conduct a comprehensive study of the fate of Cd in macroinvertebrate populations under natural conditions one must conduct complete life history studies for each group of organisms comprising the community. This requirement rapidly makes the amount of time and effort required to conduct such a study on a large scale prohibitive. Therefore, what is presented is a genral overview of several taxa in terms of Cd levels accumulated and eliminated, with more specific data for several taxa (Diptera: Chironomidae and Odonata: Anisoptera; Libellulidae).

The taxonomic groups for which the most data are available are: Ephemeroptera; Odonata, Anisoptera (Pantala hymenea) and Zyoptera (Ischnura sp.); Coleoptera (Dytiscidae); and Diptera, Chironomidae and Ceratopoganidae (Bezzia or Probezzia). Cadmium accumulation and elimination results for these taxonomic groups are presented in tabular form (Tables 11 and 12), as mean values calculated using all samples analyzed in each taxa for the periods during and after Cd inputs. This method of presentation for uptake and elimination data is probably inappropriate due to its disregard for potentially important seasonal and developmental shifts in susceptibility, uptake, and excretion of and potential for increasing Cd accumulation over time. Therefore, Tables 11 and 12 are presented only as a generalized overview. Many of the potentially confounding problems affecting our findings will be discussed in relation to specific observations made on midge or dragonfly data.

Generalized Cd accumulation data (Table 11) suggests that aquatic insect nymphs and adults do accumulate Cd and that accumulation is related to exposure concentration in many cases. Fowler and Benazoun (1974) have previously reported a direct proportionality between uptake rate and environmental concentration for Cd in the shrimp Lysmatai seticaudata and the mussel Mytilus edulis, however in our study, this relationship for aquatic insects is less exact. Those taxonomic groups obtaining the greatest Cd concentrations were the midges, mayflies and damselflies. Our findings also suggest there is no biomagnification of Cd with increasing trophic level. Detritivores and/or herbivores always maintained higher Cd levels than did carnivores regardless of treatment. Schwimer (1973) has previously reported the biodiminution of Cd in tidal invertebrates, from herbivores to predators. However, these reports from aquatic communities appear to be just the reverse

TABLE 11. MEAN CD CONCENTRATIONS IN INSECTS, DURING THE PERIOD OF CD INPUTS

Taxa	Treatment µg/l Cd	µg/g Cd dry wt	Range low	Range high	Number of Samples	Number of Organisms
Ephemeroptera *(detritivores herbivores)	0	1.6	0.0	5.8	16	18
	5	40.7	0.0	96.8	4	4
	10	176.0	59.0	324.8	8	11
Odonata Anisoptera *(Carnivores)	0	2.6	0.0	5.7	24	24
	5	18.4	9.3	38.3	7	8
	10	34.3	1.9	188.4	10	10
Odonata Zygoptera *(Carnivores)	0	3.2	0.0	11.0	33	34
	5	32.4	5.7	61.1	13	15
	10	46.4	29.5	93.4	7	9
Coleoptera *(Carnivores)	0	0.8	0.0	2.6	9	7
	5	4.1	1.2	9.3	7	7
	10	13.0	6.1	25.6	4	4
Chironomidae *(detritivores herbivores)	0	5.6	1.2	64.7	36	391
	5	55.4	17.0	190.2	40	421
	10	91.6	22.4	345.5	44	491
Ceratopogonidae *(preditors & scavengers)	0	2.0	0.0	5.6	9	52
	5	23.4	7.7	56.9	7	41
	10	33.1	11.6	56.1	8	49

*indicate a genral classification of trophic categories for dominant organisms occurring in taxonomic groups collected in our experimental system.

TABLE 12. MEAN CD CONCENTRATIONS IN INSECTS DURING THE PERIOD AFTER CD INPUTS WERE TERMINATED

Taxa	Treatment µg/l Cd	Treatment µg/g Cd dry wt	Range		Number of Samples	Number of Organisms
			low	high		
Ephemeroptera *(detritivores & hervivores)	0	-	-	-	-	-
	5	-	-	-	-	-
	10	11.1	-	-	1	1
Odonata Anisoptera *(Carnivores)	0	-	-	-	-	-
	5	-	-	-	-	-
	10	-	-	-	-	-
Odonata Zygoptera *(Carnivores)	0	4.5	0.0	13.2	17	17
	5	26.0	3.0	44.2	17	17
	10	32.4	19.3	67.8	8	8
Coleoptera *(Carnivores)	0	0.6	0.2	1.0	2	2
	5	7.4	0.1	24.5	5	5
	10	24.6	0.7	45.2	7	7
Chironomidae (detritivores & herbivores)	0	4.9	1.6	12.1	7	71
	5	31.5	6.4	107.2	17	300
	10	52.6	10.1	158.7	41	404
Ceratopogonidae (preditors & scavengers	0	5.1	1.6	9.6	3	6
	5	28.1	21.1	33.7	6	22
	10	33.8	10.1	64.1	7	18

*indicate a general classification of trophic categories for dominant organisms occurring in taxonomic groups collected in our experimental system.

for terrestrial invertebrate communities. Skinner et al. (1976) have reported the biomagnification of Cd in the terrestrial food webs of a coal ash basin.

Our results also indicate a wide range of variability in the "ability" to accumulate Cd both among and between taxonomic groups at any particular time. Similar findings have been reported by Thorp and Lake (1973, 1974) Nehring (1976) Nammingea and Wilhm (1977) Bryan (1976) and Renfro et al. (1974). Bryan (1976) states that although rates of uptake may be related to the external concentration, there is no certainty that concentrations in the organism will reflect those of the environment. Many researchers have attributed this variability to some combination of: 1) fluctuations in uptake and/or excretion rates; 2) external surface contamination, or 3) the presence of gut contents in organisms sampled. Elwood, Hildebrand and Beauchamps (1976) have reported that gut contents in Tipula sp. comprise approximately 22.1% or an individual's dry weight and over 50% of the total body burden in 70% of the 30 elements they analyzed. They concluded that gut content was an extremely important source of potential error and should be of primary considerations when determining body burdens and conducting studies on trophic level transfers of elements in aquatic systems. The potential for surface contamination of biological samples and its effect on the variability of determinations has been alluded to by numerous authors as resulting from inadequate or prolonged rinsing of samples prior to digestion.

Generalized Cd elimination data (Table 12) indicate that Cd is eliminated from the insect community over time for those populations or taxa analyzed. However, due to the variability of the data resulting from: 1) sporadic collection, 2) variable sample size, 3) effects of life cycle development, and 4) new organism recruitment; little can be said about the rates at which elimination may occur. Data suggest that of those taxa analyzed, the Coleopterans and Ceratopoganids may have reacted somewhat differently to the chronic Cd exposure than did other taxa.

Coleopteran data suggest that these organisms continued to accumulate Cd even after Cd inputs were terminated. This result is probably an artifact created by the relatively long life cycle of these organisms (in comparison with other taxa sampled) and the method by which the mean values were calculated. As previously mentioned this method of pooling samples over long time intervals does not account for Cd accumulation over time. Thus organisms with extensive Cd exposures were included in the Cd elimination data, while many earlier collected organisms with limited Cd exposures are included in the Cd accumulation data, giving the appearance of continued Cd accumulation after Cd inputs had been terminated.

Ceratopogonid Cd body burdens did not decrease after Cd inputs were stopped, even though other community components displayed a fairly rapid elimination of Cd after inputs were terminated.

Pooled monthly samples of chironomid Cd concentrations suggest a tendency for significant differences in Cd accumulation between treatment and control situations. Results of Scheff's S procedure (Kirk, 1968) for the separation of means (Table 13) indicate that even though there are significant differences in chironomid Cd body burdens between treatments and controls, there is considerable similarity in Cd levels between treatments, thus indicating that, in the case of chironomids, the fates of 5 μg/l and 10 μg/l Cd exposure are not significantly different considering the natural variability encountered.

Cadmium accumulation by chiromonoids appears extremely dependent on the time of year during which samples are collected. Mean chironomid Cd concen-

TABLE 13. SCHEFF'S S-PROCEDURE VALUES FOR INSECT CADMIUM CONCENTRATIONS MEANS AT EACH SAMPLING PERIOD

Period (month)	Treatment		
	10ppb	5ppb	0ppb
1976			
June	183.17a	142.92a	2.98b
July	218.03a	119.13b	2.26c
August	97.97a	58.57a	31.20a (questionable sample)
September	40.33a	23.08b	1.30c
October	38.84a	16.55b	2.23c
November	28.50a	26.64a	2.01b
December	40.94a	40.53a	3.84b
1977			
January	37.42a	32.85a	2.08b
February	44.28a	33.66ab	2.56b
March	52.07a	35.40a	2.55a
April	113.66a	58.58a	-
May	87.10a	66.63a	3.00a
June	32.10a	16.66ab	7.47b

Means with the same superscript within a sampling period are not significantly different ($\alpha = 0.05$).

trations calculated on a monthly basis (Figure 25) indicate that during the period of April through July, Cd burdens were higher than at any other time of year. This finding occurred on an annual basis both during and after Cd inputs were terminated, suggesting a seasonal shift in the uptake or availability of Cd in the chironomids. Thorp and Lake (1974) reported indications of potential seasonal differences in Cd toxicity to Paratya tasmaniensis (Decapoda: Atyidae). Clubb, Gaufin and Lord (1975) have reported findings which indicate that organisms collected and treated after November were less sensitive to Cd toxicity than organisms collected and treated prior to November, suggesting that early developmental stages in insects are more sensitive to Cd.

In an attempt to discern if the higher Cd body burdens observed during the April through July period resulted from: 1) Cd accumulation over time,

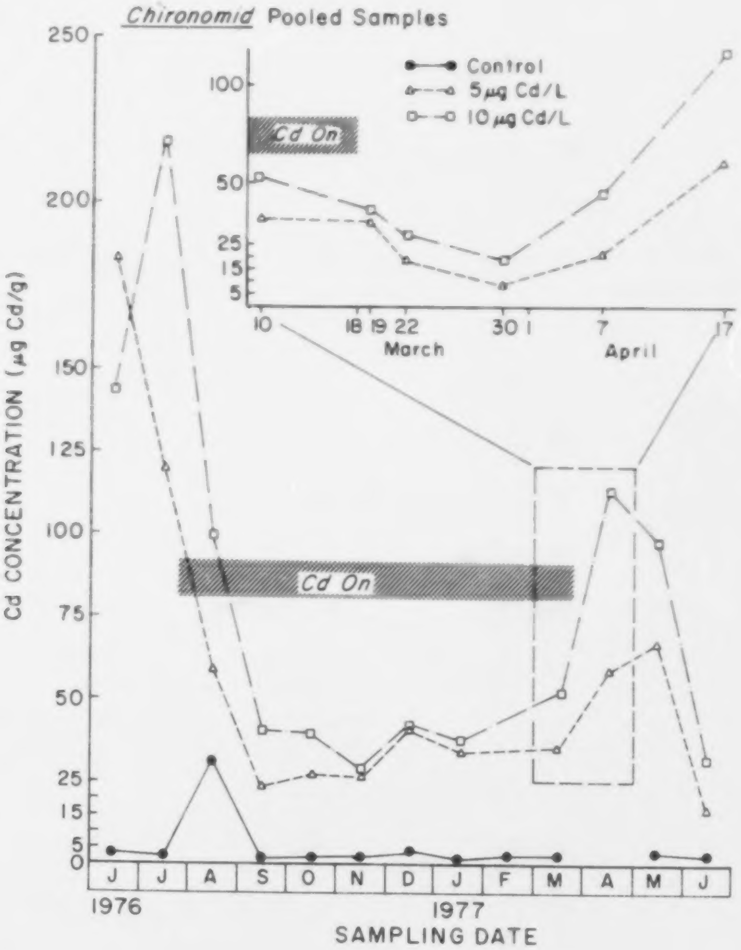


Figure 25. Mean Cd concentrations in pooled samples of chironomids, expressed on a dry weight basis.

2) shifts in surface to volume ratios, or 3) increased susceptibility to Cd of early instars; chironomid population, biomass and Cd accumulation data were used conjunctively.

The mean numbers of chironomids (Figure 26) indicates that during the period April through July, when Cd body burdens were highest, the greatest number of collectable organisms was also present. When combining the chironomid mean numbers data with mean individual dry weight data (Figure 27) one finds that not only are the greatest numbers of organisms present during April through July but that the individuals present are the smallest found during the entire year. These results indicate that during the April through July period chironomid populations are predominately comprised of early instar individuals. Thus, the potential for increased Cd accumulation over time due to extended periods of Cd exposure is eliminated.

Linear regressions using Cd concentration and mean individual dry weights were performed in an attempt to discern if the high Cd concentrations observed during the April through July period resulted from shifts in surface to volume ratios of different size individuals or from increased susceptibility to Cd of early instars. The hypothesis being that Cd is a non-regulated metal absorbed from solution by passive diffusion across a gradient created by adsorption at the surface (Bryan, 1976), and one would expect shifts in surface to volume ratios to influence the Cd level found in the organism.

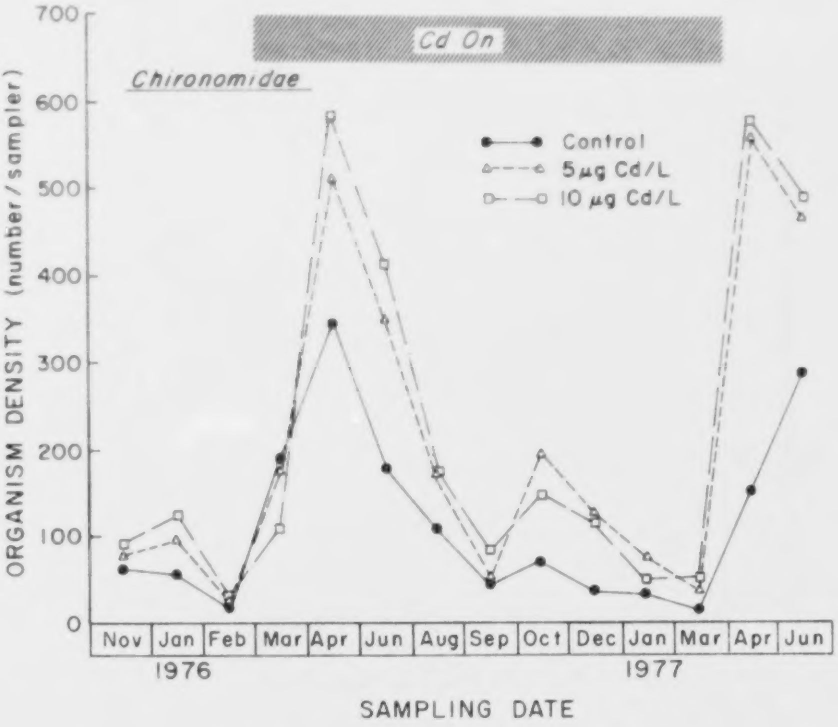


Figure 26. Density of chironomids in plate samplers.

Therefore, smaller individuals (early instars) having a greater surface to volume ratio would be expected to exhibit higher Cd levels than larger individuals (later instars) possessing smaller surface to volume ratios. Results of linear regressions between Cd concentration and mean individual dry weight (a measure of size) were not significantly different from zero, suggesting size and probably the external complexation properties of cutical were not of primary importance in controlling Cd accumulation in chironomids. These results tend to support the finding of other researchers that Cd susceptibility is apparently increased in early developmental stages of aquatic insect. Which in turn opens the door to a myriad of potential hypotheses which need to be investigated. Some of the potential hypotheses worthy of consideration in attempting to explain our findings are:

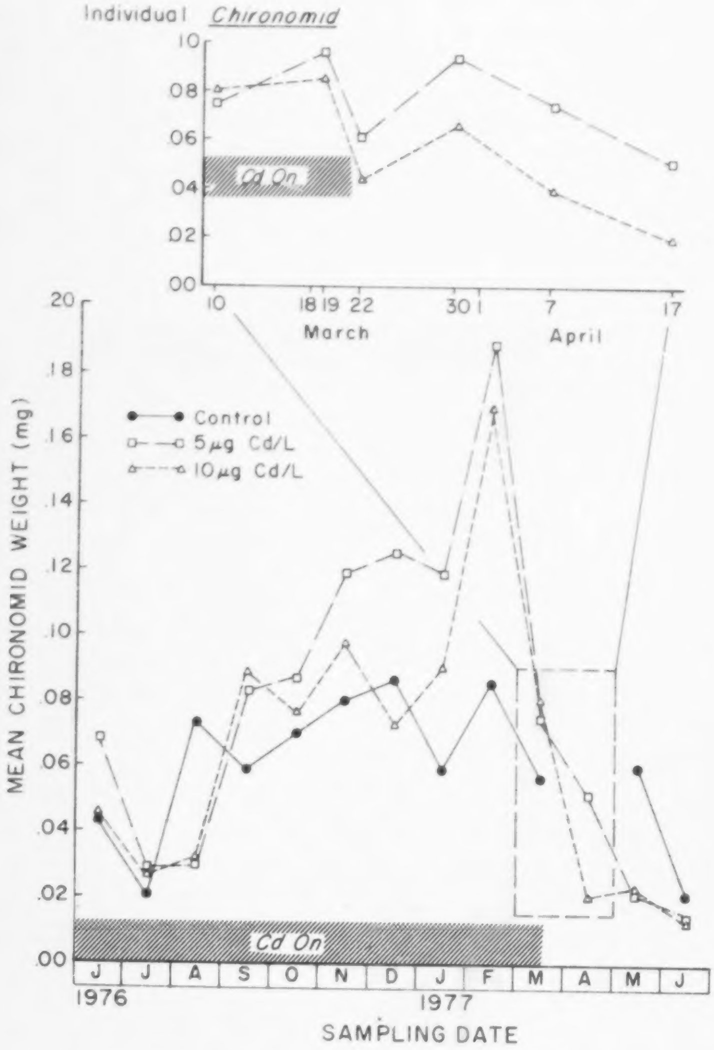


Figure 27. Mean chironomid weights.

1) Cummins (1973) statement that earlier instars of herbivore-detritivore type organisms rely primarily on detrital feeding. This means that the potential of selective feeding habits in various life stages of the same organism could play an extremely important role in controlling the concentrations of metals detected. 2) Bryan's (1976) statement that the permeability of various species is of considerable importance in determining their tolerance to metals. This statement is already supported in the literature by the findings of Renfro _et al_. (1974) who found that some species of crustaceans were more susceptible to toxicants shortly after molting than later in any particular life stage. This finding suggests that not only should a researcher consider the exposure time an organism has had to a particular toxicant but the number of molts or instars completed and the time elapsed since the most recent molt prior to collection, when trying to determine the fate of metals in macroinvertebrate populations. Also of importance here is that during early stages of development, molting generally occurs more rapidly and therefore may increase the interval of susceptibility there by increasing the Cd uptake during this segment of development. 3) Oliver's (1971) statement that the period of rapid growth of larval chironomids of univolline species occurs during the warm period of the year. If one then assumes that during periods of rapid growth the period of maximum enzyme activity occurs and combines this with Brown's (1976) findings that heavy metals may activate enzymes or enhance their activity at low concentrations, we have another high probability mechanism which metals concentration could be controlled on a seasonal or annual basis. 4) Another possible cause for seasonal fluctuation in metals concentrations of individual invertebrate taxa in field studies results from the alterations in a population's species composition. Morrison and Steele (1977) in their work with mollusks found that species within a given taxonomic group but with different environmental habitats exhibit widely varying Cd accumulation rates. This means in our case that the changes in Cd concentrations may very well be the result of a species shift in the population. However, due to the size of organisms collected during the April through July period it was time prohibitive and impossible to conduct species identifications. Our chironomid Cd data in conjunction with the finding of Morrison and Steele (1977) previously mentioned indicates that if one is interested in investigating the fate of a metal in the natural situations it becomes very important to know not only who comprises the community but also their habitats and habits.

Cadmium concentration measurements for the dragonfly _Pantala hymenaea_ indicate that this organism accumulates Cd during portions of the life cycle. Cadmium concentrations determined for each life cycle segment (adult, exuvium, nymph, and estimated nymph) for the two treatments utilized are presented in Table 14. Cadmium concentrations in adult dragonflies are proportional to treatment concentrations to which they were exposed, however orders of magnitude greater. Important to note here is that Cd levels reported for _P. hymenaea_ represent incorporated Cd, because of the manner in which these organisms were collected they had no opportunity for adulthood Cd exposure. Therefore, the values shown in Table (14) for adults could be considered as that segment of the Cd mass balance leaving the system due to individual _P. hymenaea_ emergence. However, the assumption should not be made that similar values could be extrapolated based upon individual weight for other taxons or species in the aquatic system.

TABLE 14. MEAN CD CONCENTRATIONS FOR P. HYMENAEA LIFE CYCLE SEGMENTS BY TREATMENT EXPRESSED ON A DRY WEIGHT BASIS ($\bar{X}$ ± 2 SE).

Segment	N	5ppb	N	10ppb
Adults	4	1.6 ± 0.4	15	3.2 ± 0.7
Exuvia	5	23.8 ± 10.3	28	33.9 ± 5.3
Nymphs	6	17.3 ± 8.9	8	19.1 ± 6.3
Estimated Nymphs	4	5.2 ± 2.2	15	8.7 ± 1.1

Cadmium data based on analysis of final instar exuvia indicate that on a per gram dry weight basis the exuvium of P. hymenaea has a higher Cd content than any other segment of the life cycle analyzed (Table 14). However, Cd levels accumulated do not appear proportional to treatment. Results indicate a trend towards increasing Cd concentrations over time along with a decreasing trend in exuvium dry weight over time, suggesting that surface sorbtions may be the means of Cd accumulation by the exuvia as Bryan (1976) has previously suggested. However, linear regressions performed between Cd concentration and individual exuvium dry weight were not significantly different from zero, suggesting that some other mechanism or mechanisms are involved in affecting the accumulation of Cd by the exuvium. Many of these potential mechanisms have been alluded to in earlier segments of the macroinvertebrate discussion.

Cadmium data for P. hymenaea nymphs is based on a relatively small sample size of highly variable Cd determinations. Results reported in Table (14) indicate that the nymphs do accumulate Cd and that this accumulation is not proportional to treatment. However, it should be noted that all nymph Cd determinations were accomplished using whole organisms from which the gut contents were not removed. Therefore, variable amounts of food containing Cd probably has greatly influenced the variability of these samples.

Cadmium data for estimated nymphs derived by combining the µg Cd/ml sample for adults and their respective exuvium cast off at emergence then dividing by the combined dry weight of those same samples, suggests that Cd accumulation is proportional to treatment and is considerably different than levels determined for actual nymphs. The difference observed between actual and estimated nymphs may very well represent the percentage of total Cd body burden attributable to gut contents in P. hymenaea. However, further research is required before any specific conclusions can be drawn.

While calculating the total Cd body burdens for estimated nymph values, the percentage contributed by both adults and exuvia was also determined (Table 15). Results indicate that there is no difference in the percentage

TABLE 15. MEAN % CD IN EACH LIFE CYCLE SEGMENT OF ESTIMATED NYMPHS BY TREATMENT ($\bar{X}$ ± 2 SE)

	5ppb		10ppb		Combined	
Segment	N		N		N	
Adults	4	32.3 ± 17.2	15	32.0 ± 6.1	19	32.1 ± 5.8
Exuvia	4	67.7 ± 17.2	15	68.0 ± 6.1	19	67.9 ± 5.8

of the total Cd body burden attributable to adult or exuvium, with increasing treatment level. This finding suggests that the mechanisms of accumulation and elimination are constant and not altered by treatment level (at least at chronic levels). These data also bring to our attention the ability of the exuvium (exoskeleton) to accumulate Cd and the potentially important roles this structure may play in the toxicity and/or cycling of Cd.

The ability of the exuvium of P. hymenaea to accumulate approximately 68% of the total Cd body burden is similar to literature values for other organisms. Renfro et al. (1974) reported that 45% of the total Zn^{65} body burden of shrimp was located in the exoskeleton while approximately 61% was found in the exoskeleton of crabs. Renfro et al. (1[illegible]4) concluded that the occurrence and rate of molting in invertebrates could account for a considerable portion of the variability of their and other researchers studies and that exoskeletons are of potential importance in the cycling of metals in the environment, either through their actions as a metals sink or by adding in the recycling or availability processes. Another question to be proposed and investigated in relation to the Cd accumulating abilities of the exuvia P. hymenaea is: does the exuvium act as a mechanism protecting a species from Cd toxicity due to its ability to accumulate or absorb the metal? There is a significant amount of literature on mechanisms in other invertebrate forms which have the ability to complex metals and which have been hypothesized as mechanisms for transporting and potential detoxifying metals. Bryan (1976) lists several: 1) the ability of blood proteins to bind Zn in crayfish, 2) the apparent storage of Cu in fine granules within the epidermal cells of marine polychaeles, and 2) the presence of wandering leucocytes in mollusks and their known importance in transporting and detoxifying metals. The possibility does exist that the exoskeleton may be functioning in a similar manner for P. hymenaea.

Population and Community Effects

Macroinvertebrates collected from the experimental channels were tolerant forms, typical of pond or sluggish waters (stream margin and littoral zone) in the southeastern United States. The various benthic sampling methods utilized during the 23 month study collected a total of 53 different taxa of which only 14 were collected routinely. Macroinvertebrates consisted primarily of numerous chironomid species, mayflies, *Callibaetis* *sp*. and *Caenis* *sp*., damselfly, *Ischnura* *sp*. and two genera of Ceratopogonidae, *Dasyhelea* *sp*. and *Bezzia* *sp*. or *Probezzia* *sp*. Also present, but less abundant, were several species of Anisoptera, Hemiptera, Coleoptera, Trichcoptera, Lepidoptera and Annelidae. Appendix II lists all macroinvertebrates collected from the treatments during the study period and the method of collection. Macroinvertebrate colonization continued throughout the study period with the continual recruitment of new species. Sampling emphasis was placed on insect fauna, resulting in the possible omission of some non-insect invertebrates.

Macroinvertebrates which colonized the channels were primarily insects adapted for invading newly created bodies of water by flight. Benthic invertebrate community development was allowed to proceed naturally. Therefore, only a few organisms were collected from the channels prior to the establishment of the periphyton community. Unlike most woodland or pastoral streams where the dominant energy source results from allochtanous inputs creating the development of heterotrophic systems, our artificial channel system is highly autotrophic relying on periphyton and filimentous algal forms as the energy basis for the establishment of higher trophic levels. Because of this autrophic status and the physical structure (current velocity, water temp., sand substrate, etc.) one would not expect to find a number of macroinvertebrate forms whose physiological or morphological development and/or behavior has specialized them for the roll of processing larger organic material (leaves, macrophytes, etc.) converting it into partial sizes and textures required by other components of the invertebrate community. Such organic processors such as Trichoptera, Plecoptera and some of the Ephemeroptera, Coleoptera and Diptera were rarely collected during this study.

Those organisms first to establish permanent populations in the channels were the midge larvae, which are known to dominate sandy substrata, folowed shortly by a limited number of a variety of other organisms. The most important of these initially rare taxa were: *Pantala* *hymenaea*, with a short life cycle and common to temporary ponds (Corbet, 1962); *Hesperocorixa* *sp*., one of the corixids, which as a group are acknowledged to be partially responsible for the primary conversion of plant materials into animal food (Usinger, 1971) and *Callibaetis* sp., a mayfly found in small temporary woodland ponds (Burks, 1953). These initial colonizers were followed sporadically by other organisms throughout the study, of which the mayfly *Caenis* sp., damselfly *Ischnura* sp., dragonfly *Erythrodiplax* *miniscula* and the biting midges *Dasyhelea* sp. and *Bezzia* or *Probezzia* sp. were the most important.

Colonization patterns observed in the channels were similar to those observed in natural aquatic systems (Egglishaw, 1964; Hynes, 1970). Populations increased rapidly in late February or early March and peaked in April, due to newly hatched, early instars. This large population, then, gradually diminished through the summer due to predation, natural mortality and emergence. In October there was a slight increase in population levels resulting from ovaposition. However, no significant alterations in this colonization pattern could be attributed to Cd.

The density of the entire macroinvertebrate community (Figure 28) although a crude method of representing benthic community responses (Pennak and Van Gerpen, 1947), indicated that with the exception of two points, September 1976 and April 1977, there were no significant differences among Cd treatments. The September 1976 difference resulted from a tremendous increase in the Pristina aequiseta populations in control channels, probably due to an increase in dead and decomposing organic material created by the breaking-up of filimentous algal mats covering the channels. The April 1977 difference resulted from extremely rapid recruitment of new midge larvae in the channels previously receiving 5 and 10 μg/l Cd as opposed to the slower recruitment into control channels. This phenomenon may be due to a number of phenomena including: differences in density or structure of other invertebrate populations, algal or macrophyte community colonization, or visual preference in the selection of ovapositation sights as has been reported for some aquatic insects.

Significant differences in the number of taxa colonizing multiplate samplers attributable to Cd treatments occurred in only 3 of 14 sampling periods (Figure 29). In all three cases, control channels had significantly more taxa colonizing them than those receiving either 5 or 10 μg/l Cd. Although the number of these significant differences were few, they occurred in

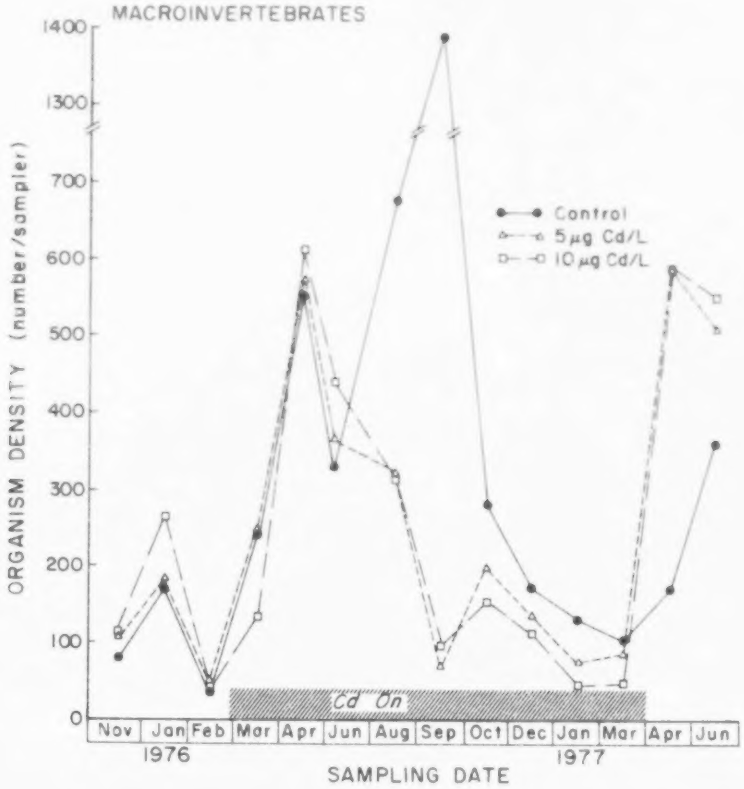


Figure 28. Mean number of macroinvertebrates per sampler.

succession during Cd inputs, and at a particularly important time in the colonization pattern, late fall and winter. This period of the year is when the benthic fauna is generally most stable in both density and diversity. Also of importance is that those individuals over-wintering in the system are responsible for initiating the following springs recruitment and colonization. Thus any type of effect which acts in an additive fashion with natural seasonal affects to affect the size and composition of the over-wintering benthic community could seriously affect colonization and successional development in years to come. The number of taxa in control channels (Figure 29) did not fluctuate as sharply as those in channels receiving 5 or 10 μg/l Cd inputs, indicating control channels were more stable and did not respond as rapidly to changes in environmental conditions as did channels receiving Cd. This phenomena is probably the result of the control channels maintaining higher algal productivities and greater macrophyte colonization during the preceding part of the year, thus building up a greater organic and nutrient base, as well as diversity of habitat. This observation is supported by our algal and macrophyte data and the works of Jones (1940; 1941; and 1958) who hypothesizes that insect larvae of a stream are largely affected by the indirect effects of heavy metals pollution and that the principal indirect effect of such pollution is the formation of unstable physical conditions due primarily to the elimination of algal and aquatic macrophytic growth.

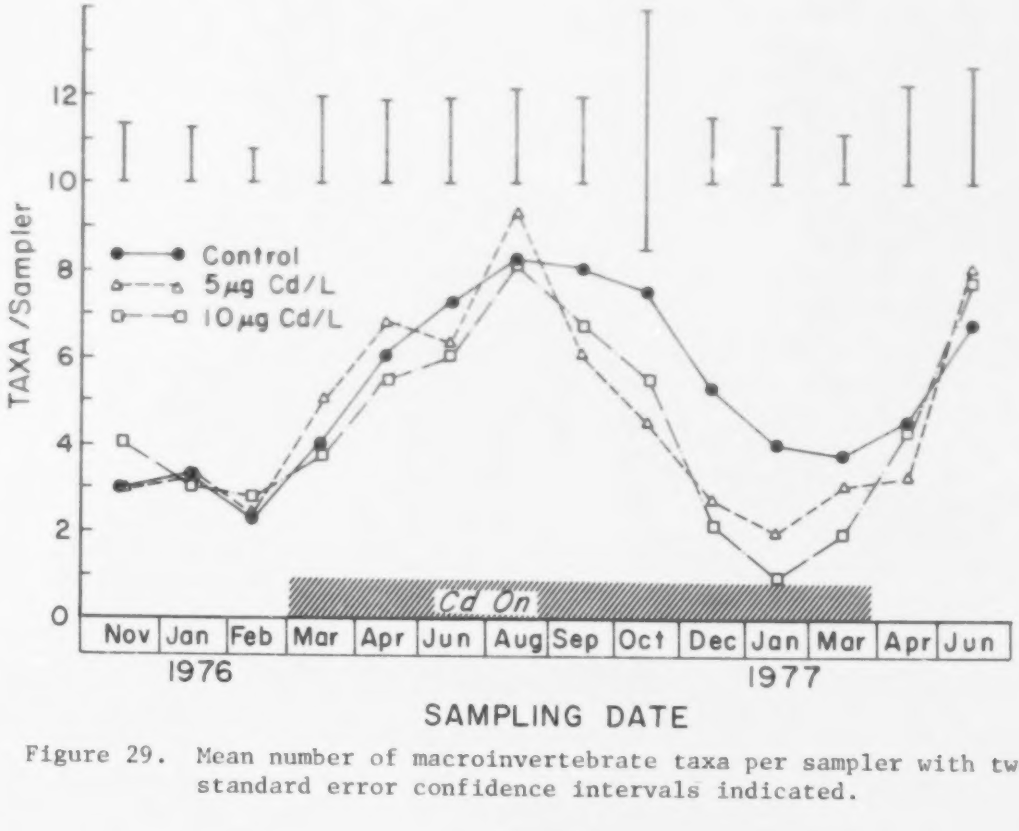


Figure 29. Mean number of macroinvertebrate taxa per sampler with two standard error confidence intervals indicated.

Although there were no significant differences in organism densities due to Cd treatments, the abundance of dominant taxa and shifts in relative community compositions revealed interesting trends. Between 88 and 100% of the macroinvertebrate communities colonizing our system was accounted for by four taxa; Chironomidae, Annelida (Pristina aequiseta), Copepoda (Euryclops agilis) and Ephemeroptera (Figures 30, 31 and 32). The greatest fluctuations in macroinvertebrate community composition resulted from shifts in the relative abundance of dominant taxa, while the relative composition of rarer taxa remained constant. This pattern was reversed in channels receiving Cd, where rarer taxa comprised a larger segment of the community. The rarer taxa in these situations fluctuated considerably, while the dominant taxa maintained more stable population levels during Cd inputs. This trend appears to have some relationship to environmental Cd concentration, with dominant taxa populations becoming more stable and rarer taxa populations exhibiting greater instability as Cd concentration increases. Stability in the dominant taxa group resulted primarily from the fact that as Cd treatment increased, Chironomid abundance also increased, thus Chironomids comprised more and more of the total invertebrate community. Our macroinvertebrate data indicated that the presence of chronic Cd pollution at the 5 and 10 μg/l level is not shown by indicator species but by the dominance of tolerant species. Our findings are supported by Hynes (1960) who states that no special fauna are indicative of heavy metals pollution, although the surviving species may be more abundant.

Population densities and percent community composition of Chironomidae, Ephemeroptera, Ceratopogonidae, Annelida, and Copepoda were affected either directly or indirectly by Cd. Chironomid population densities were always less in control channels. However, there were only two points at which this trend had mean values which were statistically different (Figure 26). This observation was primarily due to habitat availability; resulting from more periphyton covered sandy substrata and less decomposing organic material deposition in channels receiving Cd inputs. Mean weight data for individual chironomids (Figure 27) indicated that organisms taken from control channels were generally lighter than were similar individuals taken from channels receiving Cd inputs. This trend continued during Cd input and is most probably due to habitats and related environmental conditions. Oliver (1971) states that larvae of many Chironomid species have the ability to grow and develop as conditions permit. Our research, however, leaves us with no explanation as to why chironomids collected from channels receiving 5 and 10 μg/l Cd should have achieved a greater individual body weight.

Abundance of individual mayfly genera indicated a slight shift in occurrence of the genus Caenis in controls vs. channels receiving Cd (Figure 33). Caenis became more prevalent in the control channels as opposed to treatment channels during Cd inputs. It should be noted that even though the trend did occur during all sampling periods, at no time was there any significant ($P < 0.05$) differences observed. It is believed that this trend is again the result of increased algal and macrophytic growth in control channels.

Ceratopogonidae (biting midges) became increasingly more abundant in channels receiving 5 and 10 μg/l Cd (Figure 34). Increased prevalence of

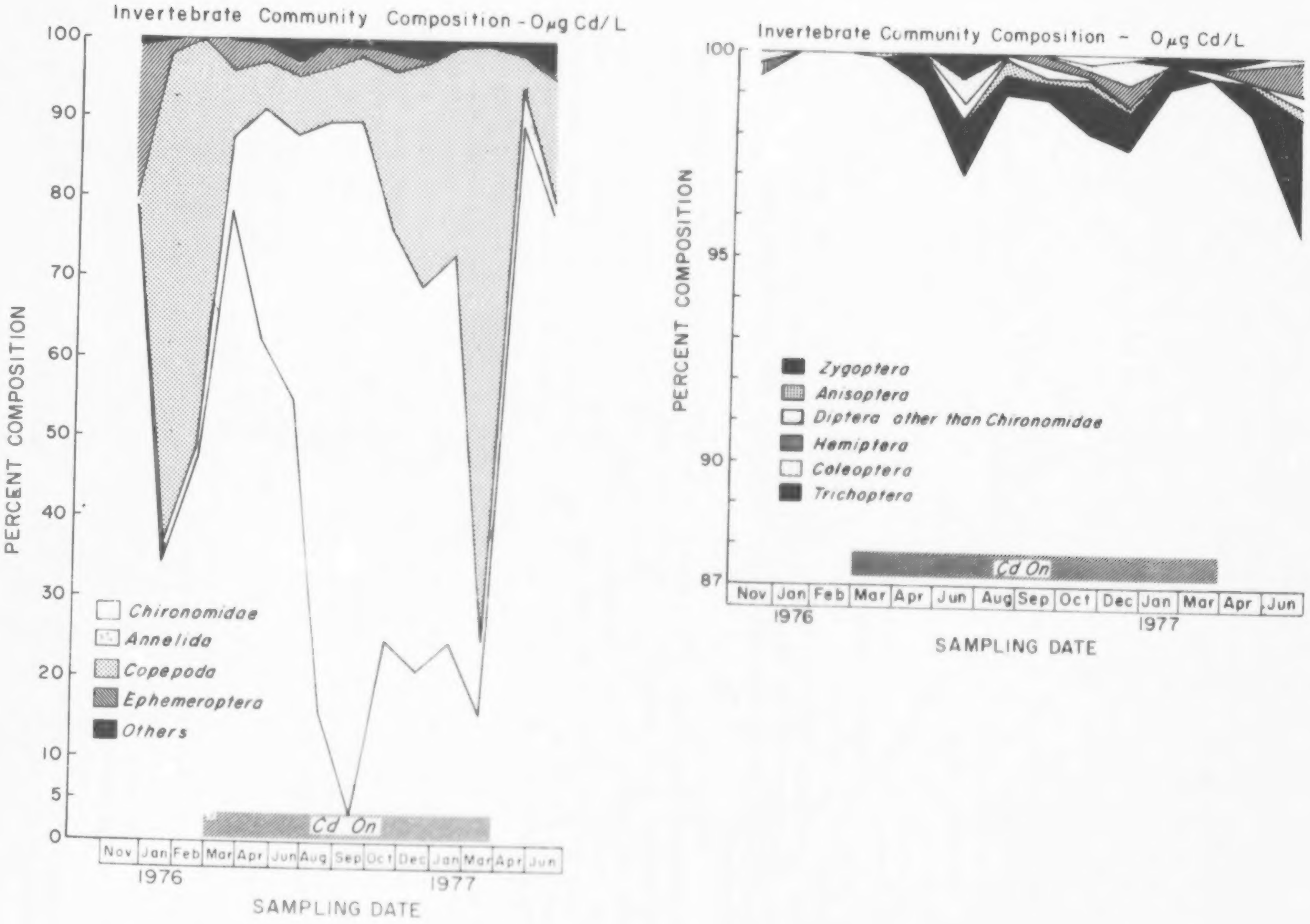


Figure 30. Percent community composition of macroinvertebrate community in control channels.

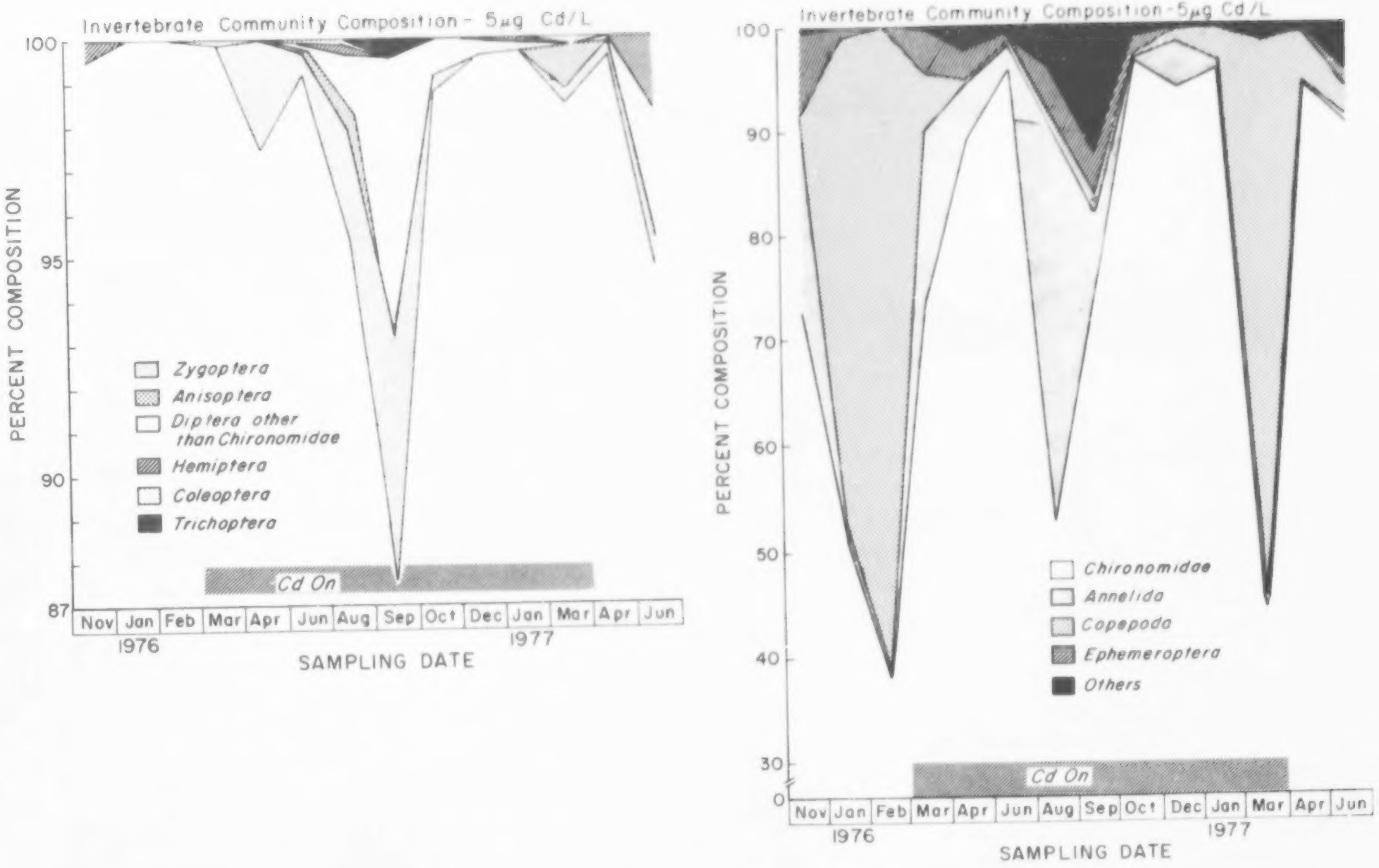


Figure 31. Percent community composition of macroinvertebrate community in channels receiving 5 µg Cd/1.

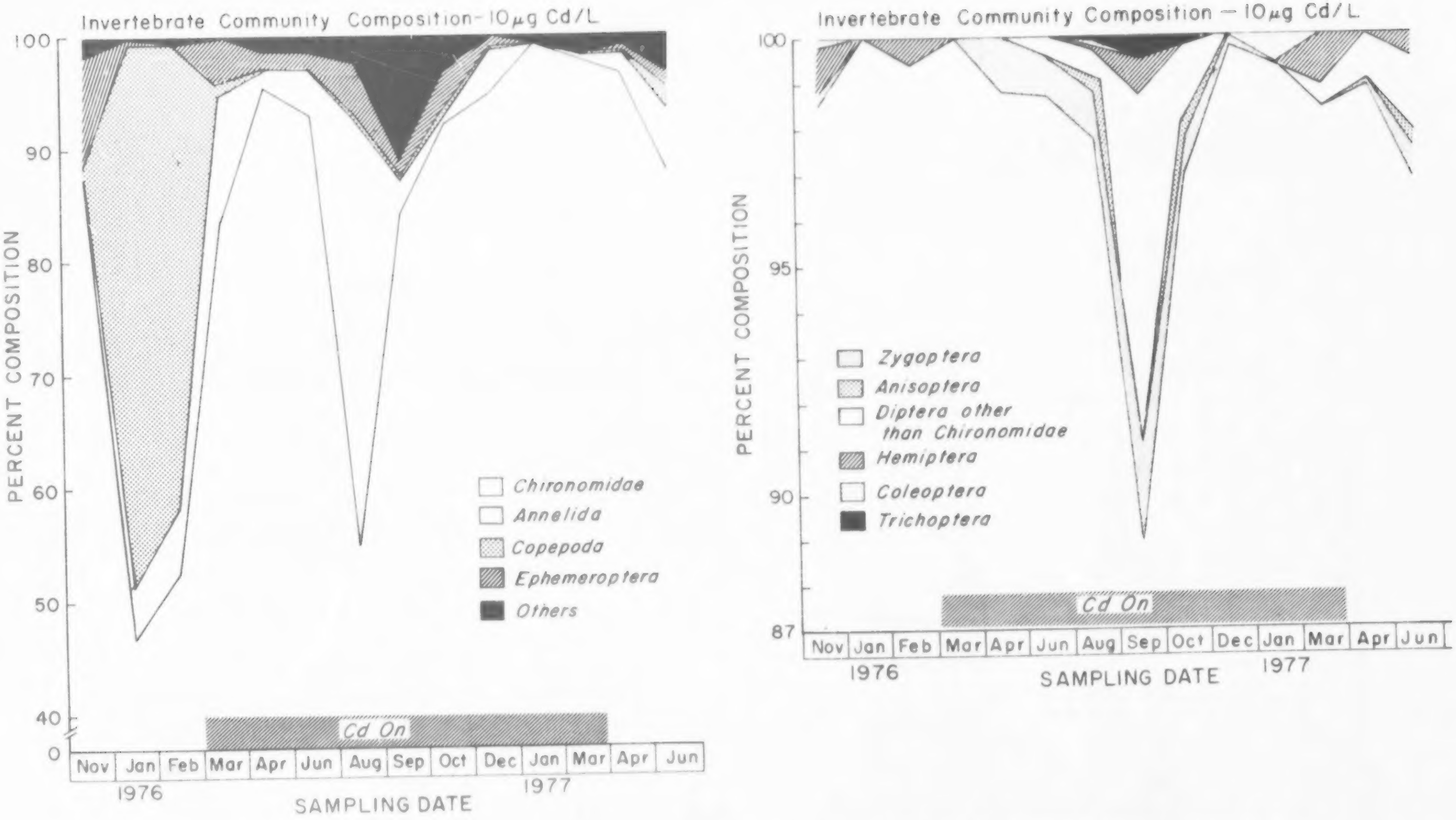


Figure 32. Percent community composition of macroinvertebrate community in channels receiving 10 µg Cd/1.

Ceratopogonidae in treatment channels resulted from the presence of one genus, Dasyhelea sp (Figure 34). Data taken from bottom sediment samples in March 1977 indicated how prevalent these organisms became in channels receiving Cd inputs. In control channels a total of 6 Dasyhelea were collected from 8 random bottom samples as opposed 484 and 661 individuals collected from similar samples taken from 5 and 10 μg/l Cd treatments, respectively. The increased Dasyhelea densities in control channels appears directly related to decreased macrophyte colonization in channels receiving Cd inputs, with a corresponding increased development of an algal mat covering the channel bottom. Thomsen (1933) in Johannsen's (1969) book on Aquatic Diptera indicates that the blanket algae of ponds is the preferred habitat of Dasyhelea sp.

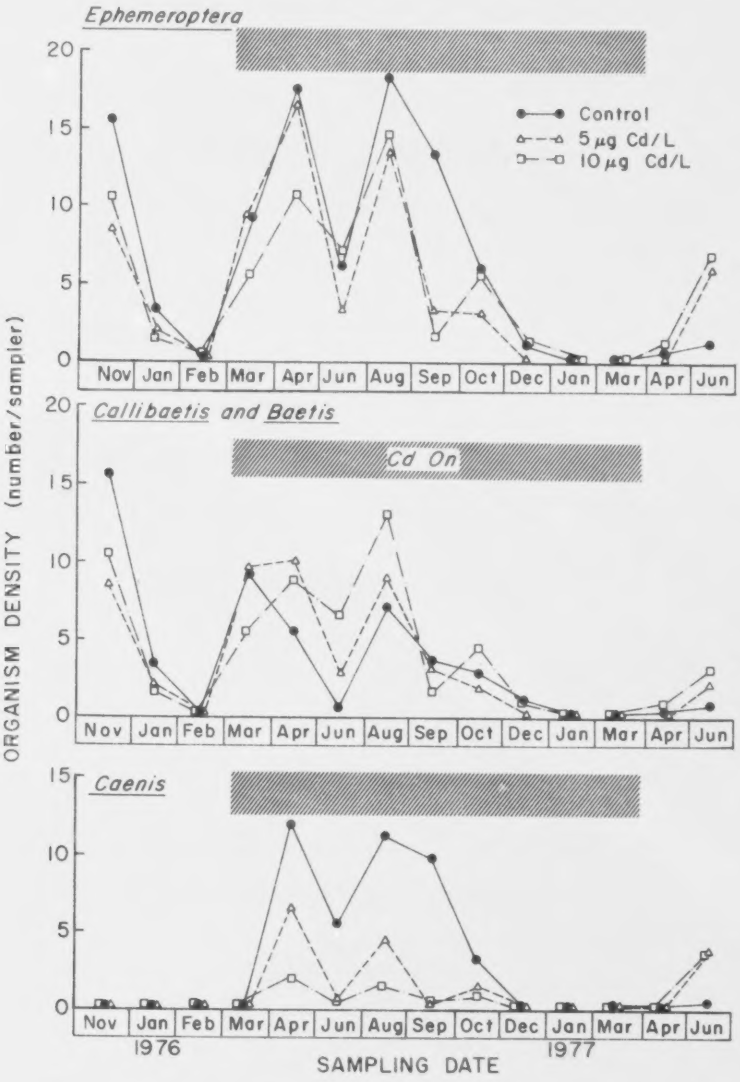


Figure 33. Density of Ephemeroptera per sampler.

Comparisons made between aquatic macrophyte biomass and macroinvertebrates abundance data, although sporadic and limited, indicated that the presence of macrophytes influenced both the number and type of organism present in many cases. Similar findings have been observed in natural aquatic systems by Egglishaw, 1963 and by Cole, 1973. Populations exhibiting distinct relationships to macrophyte colonization in our system were: 1) Dasyhelea sp., whose abundance decreased with increasing macrophytic colonization; 2) Erythrodiplax miniscula, who only began colonizing our system during the second season after substantial macrophyte colonization had occurred in the upper portion of most streams; 3) Pantala hymenaea, whose abundance gradually declined during the second season as algal and macrophytic colonization and growth expanded downstream. Therefore, any direct effect on macrophyte and/or periphyton colonization or growth attributed to Cd treatment indirectly affected the macroinvertebrate community also.

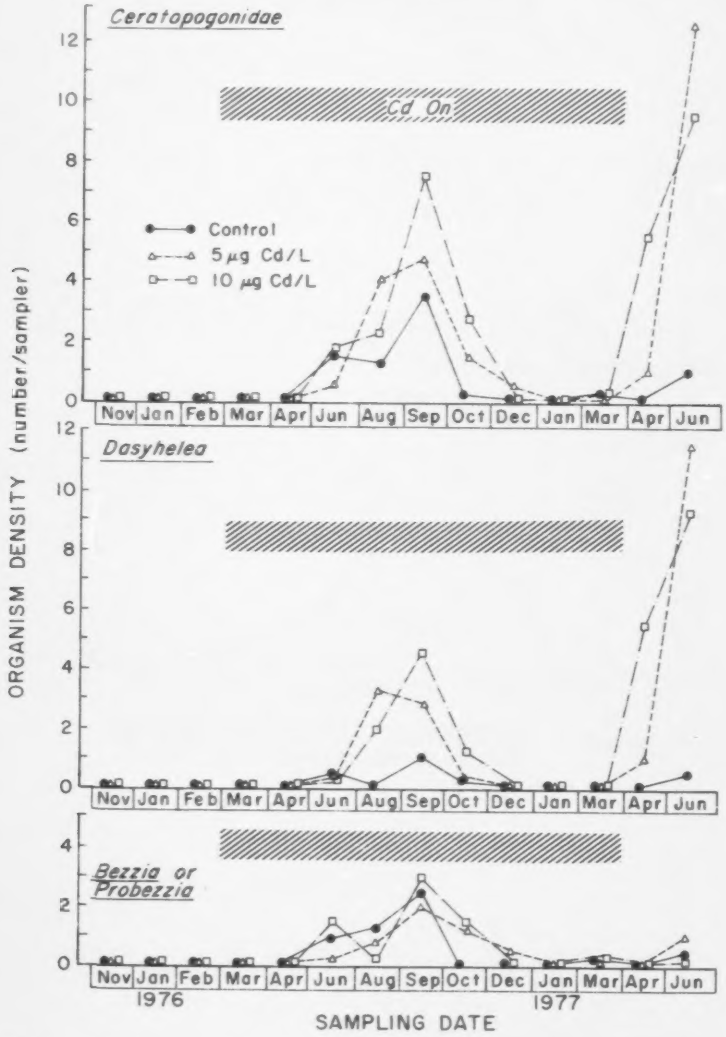


Figure 34. Density of Ceratopogonidae per sampler.

Microinvertebrates

Between 88 and 100% of the macroinvertebrate community was accounted for by four taxa; Chironomids, Annelids (Pristina sp.) Copepods and Ephemeropterans (Figures 35 and 36). The greatest fluctuations in the composition of macroinvertebrate community inhabiting the control channels resulted from shifts in the relative importance of dominant taxa, while the relative composition of the rarer taxa remained relatively constant.

Annelids (Pristina sp.) (Figure 35) and Copepods (Figure 36) displayed the most pronounced trends observed in all the invertebrates enumerated for this section of the study. These observations would most likely have been overlooked in conventional macroinvertebrate surveys or would only have been observed if additional macroinvertebrate surveys were conducted at the same time. The reason for these groups being reported in this section stems from our previously mentioned changes in sampling techniques which allowed for the collection and enumeration of these two groups.

Data on the Pristina, annelids and Copepods showed their abundances to decrease noticeably with increasing Cd treatment. Pristina populations in control channels were greater than in treatment channels (Figure 37). However, in only 2 of the 14 sample periods were the differences significant. The results of Copepod data, where again, larger populations in controls as opposed to treatment channels during the period of Cd inputs (Figure 36). In

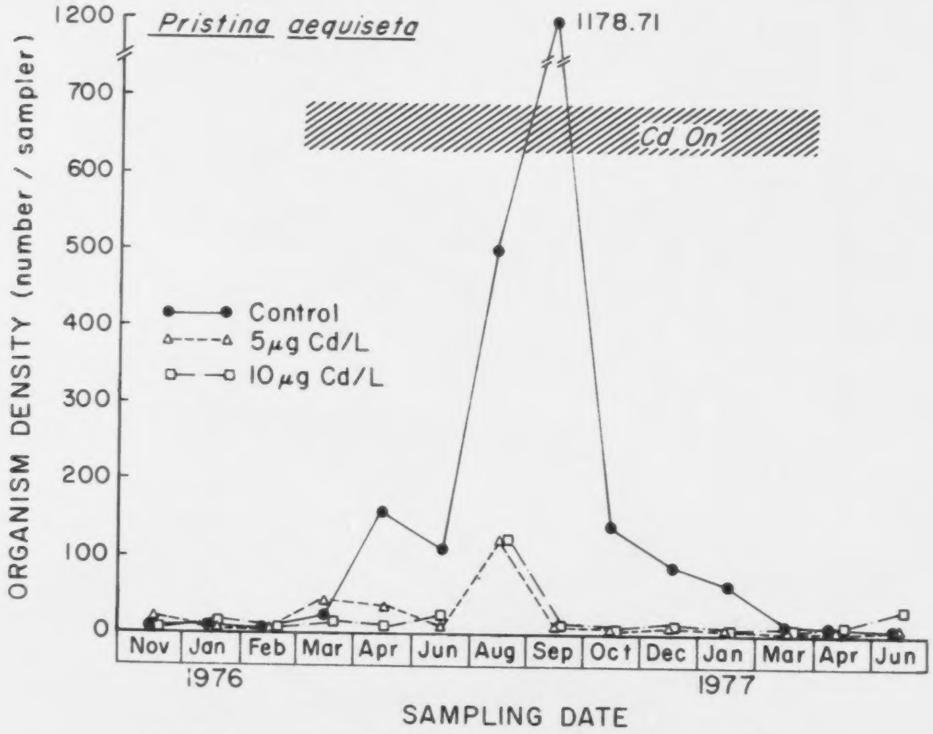


Figure 35. Density of P. aequiseta per sampler.

9 of 14 sampling periods the controls had significantly larger populations of Copepods than did treatments. Eight of these significantly different samplings occurred during the Cd input period. Additional information on these groups of organisms can be found in the microinvertebrate segment of the report.

The major groups affected by Cd were flagellated and ciliated protozoans, testate amoebae of the genus Difflugia, and ostracod and copepod crustaceans (Table 16). Significant F values demonstrate a Cd effect but do not indicate whether population densities were increased or decreased in the treated systems. Cadmium reduced populations of the two crustaceans and the amoeba Difflugia, the expected effect. However, densities of the flagellate and ciliate protozoans in the channels receiving Cd were elevated relative to those in control systems. In general the densities of microinvertebrates was

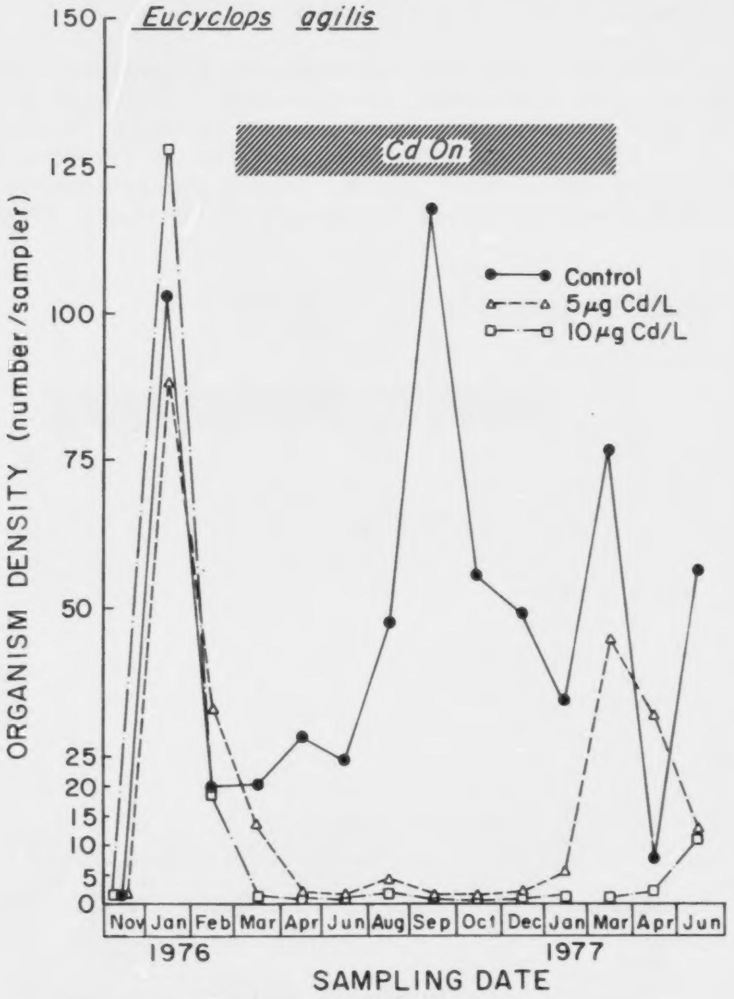


Figure 36. Density of E. agilis per sampler.

elevated in channels receiving Cd (Figure 37). Rotifer densities were also elevated in channels receiving Cd in 11 of 12 samples; however, within treatment variability was sufficiently great that only four statistically significant F values were observed. This is significant in that Buikema et al. (1974) observed that rotifers might be a convenient organism for bioassay work. The lowest Cd concentrations used in their studies, however, were much greater than those used in our study.

It is difficult to compare protozoan densities from the sponge samplers to other research work. No long term studies of the effects of metals on this group have been conducted. In general, laboratory studies have been carried out with relatively high metal concentrations and single species (Gray and Ventilla, 1973; Milles, 1976; Bergquist, 1976; Giesy et al., 1977); Lansing et al., 1977) or with high metal concentrations and simple communities (Burbanck and Spoon, 1967; Ruthren and Cairns, 1973) exposed for short time periods. The apparent stimulatory effect of Cd on ciliate (especially Paramecium barsaria) and flagellate (especially Chlamydomonas sp.) protozoans may be due to release from predation competition. Eucyclops agilis, the only copepod observed in the samples, is essentially a vegetation (Fryer, 1957)

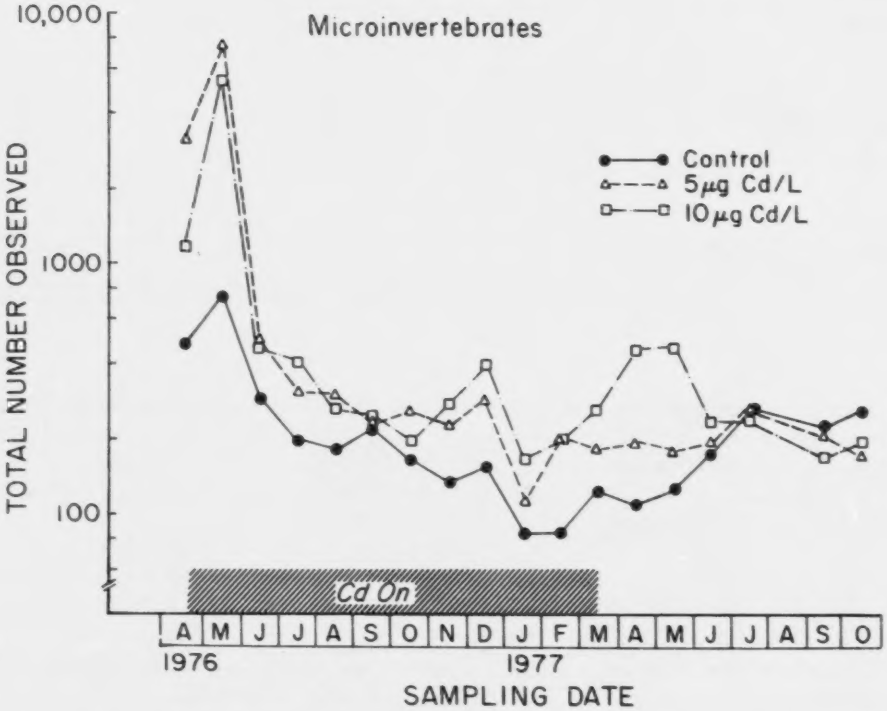


Figure 37. Total number of microinvertebrates observed per month in polyurethane sponger.

TABLE 16. EFFECT OF CD ON DENSITY OF TAXA IN SPONGE SAMPLERS.

Taxa	# of significant F values ($P < 0.05$)	# of months sufficient data available
Protozoa		
Sarcodina (excluding Difflugia)	1	12
Difflugia sp (3)	11	12
Flagellata	6	12
Ciliata (excluding Paramecium bursaria)	10	12
Paramecium bursaria	9	12
Platyhelminthes		
Turbellaria	4	11
Aschelminthes		
Rotifera	4	12
Nematoda	1	12
Annelida	1	7
Arthropoda		
Crustacea		
Branchiopoda (Alonopsis elongata)	2	12
Ostracoda	8	12
Copepoda (Eucyclops agilis)	10	12
Insecta		
Diptera (Chironomidae)	1	8

and its increased densities in the control channels may have been responsible for the reductions in Chlamydomonas populations in these control channels, but E. agilis is too small to feed on P. barsaria and rotifers. That Cd has a direct stimulatory effect, perhaps by controlling parasitic bacteria or fungi, cannot be discounted.

Macroinvertebrate community diversity and evenness were calculated using five different indices: Simpson's Index (Bowman et al., 1971), (Figure 38); evenness of Simpson's Index (Bowman et al., 1971) (Figure 39); Shannon's Index ($\bar{H}$) calculated using $\log_2$ (Figure 40); Evenness of Shannon's Index ($\bar{H}/\log_2$ (N-SPP) (Figure 41); MacIntosh's Index (Pielou, 1969) (Figure 42); Evenness of MacIntosh's Index (Pielou, 1969) (Figure 43); Probability of Interspecific Encounter (Hurlbert, 1971) (Figure 44), Evenness of Probability of Interspecific Encounter (Hurlbert, 1971) (Figure 45); and Renzi's Generalized Entropy Series, first with a = 1 then as a = 2 (Hill, 1973) (Figures 46-49). The objective of this exercise was not to compare the accuracy of these indices in distinguishing the potentially subtle effects of chronic Cd exposure, but instead to determine which of these indices might best illustrate any subtle effects which might occur. The basis by which diversity and evenness were calculated are: 1) as individual samples, the means of which are plotted by treatment and sampling period in portion A of Figures 38-46 and 48; 2) by summation, here each sample is added to a running sum and diversity and evenness is calculated on the sum, portion B of Figures 38-46 and 51 represent values calculated on the composite total of all samples collected for each treatment and sample period. The reason for calculating diversity and evenness on both basis was to see if sample size affected our ability to distinguish chronic effects.

Immediately after cadmium input was started there was a decrease in diversity in those channels, while the diversity in central channels increased, due to continued colonization (Figures 38-49).

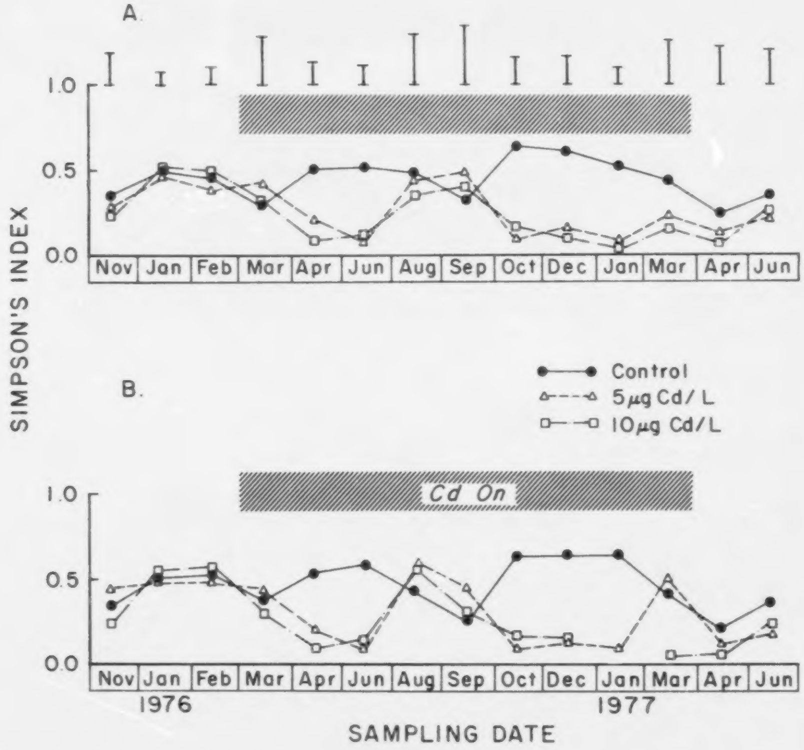


Figure 38. Simpson's diversity index. A, means calculated across sampler by sampling period with two standard error confidence intervals indicated. B, calculated by summation.

Statistically significant ($P < 0.05$) differences in diversity and evenness occurred in 5 of 14 sampling periods. These differences occurred during April through June, the spring and early summer emergence and recruitment period, and again in October through January, the fall and winter minimum population period. In all five cases the control channels had significantly higher diversity and evenness values than did channels receiving either 5 or 10 μg/l Cd. There appeared to be no significant differences in the 5 and 10 μg/l treatments. Our results indicate that significant differences occurred only during the period of Cd input and not during the three months prior to or the two months after Cd was input. Therefore, it appears that 5 and 10 μg/l Cd does affect the invertebrate community sufficiently to affect both diversity and evenness calculations. It should also be pointed that regardless of the method or index employed the results were similar with only the magnitude of the values calculated being affected.

Diversity and evenness indicate that channels receiving 5 and 10 μg/l Cd may be somewhat less stable than control channels as did the abundance and community composition data hypothesis of Jones (1940, 1941, 1958) that insects are probably indirectly affected by chronic levels of metals exposure

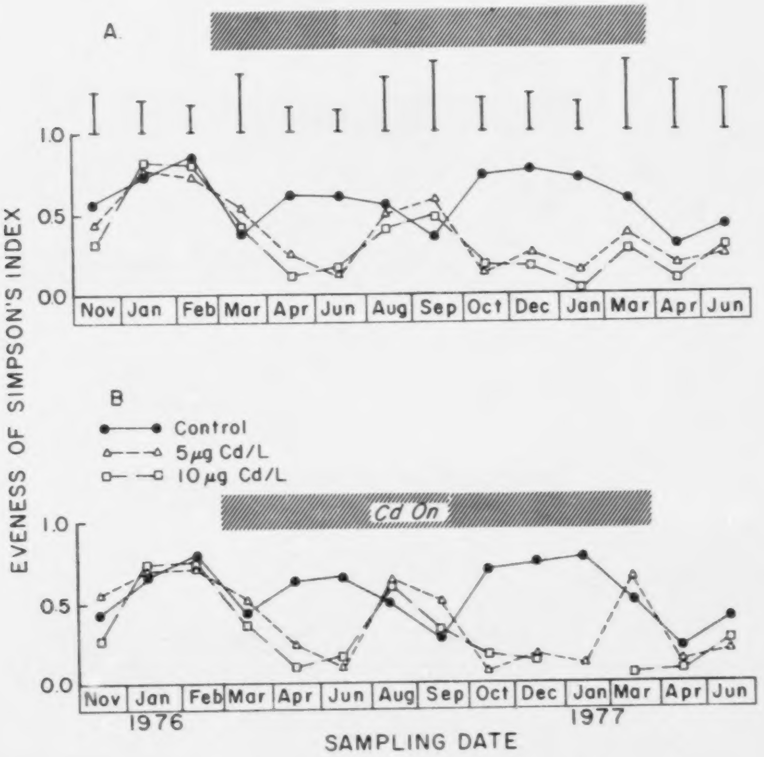


Figure 39. Eveness of Simpson's diversity index. A, means calculated across sampler by sampling period with two standard error confidence intervals indicated. B, calculated by summation.

and it is the effect on the surrounding environment which harms them the most (direct effects in algae and macrophytes etc.). In our study, what we have observed is not necessarily the effects of 5 and 10 μg/l Cd on existing algal and macrophyte communities, but instead the retarding of their successional development. Thus the 5 and 10 μg/l Cd channels were maintained at earlier successional stages than controls, and for this reason, probably were somewhat more unstable.

Peak diversity occurs approximately 30 days later in channels receiving Cd than in those receiving no Cd regardless of calculation methods employed (Figures 40-51). This is apparently the result of delayed development of individuals, with a concomitant delay in hatching and thus community structure changes.

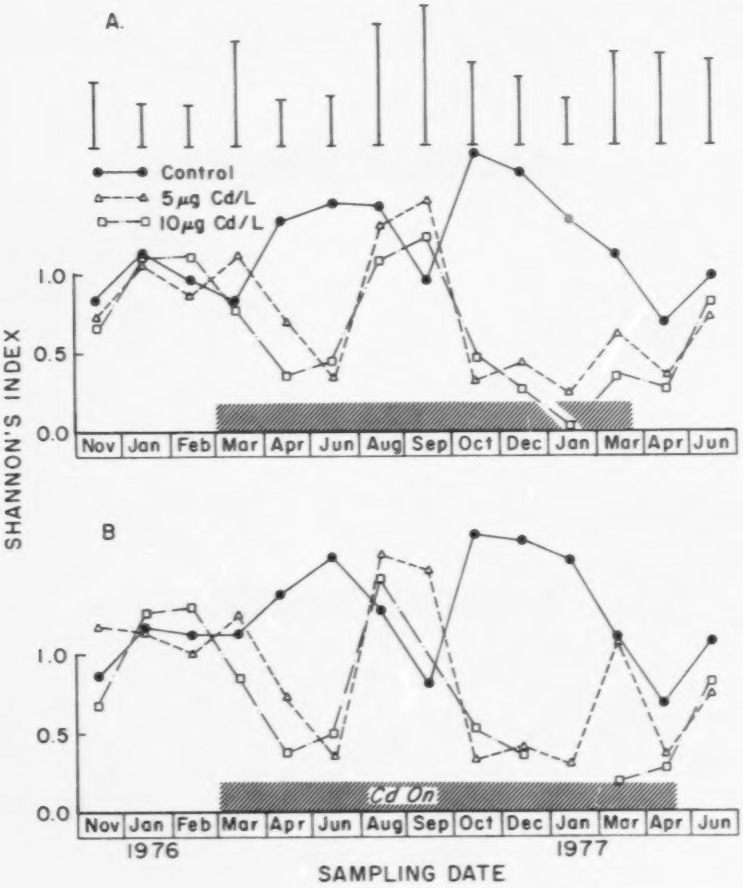


Figure 40. Shannon's diversity index A, means calculated across sampler by sampling period with two standard error confidence intervals indicated. B, calculated by summation.

Molluscs

Due to the large amount of literature available on trace metal accumulation by molluscs (Bertine and Goldberg, 1972; Wier and Watter, 1976 and Pringle et al., 1968) and their increasing use as biomonitors of heavy metals pollution, both gastropods and pelecypods were used in our study.

Two gastropods were proposed for study; the pulmonate, Helisoma trivolvus and the prosobranch Campeloma lima. Through the use of these two distinctly different physiological forms it was believed that additional information regarding uptake and biological effects of Cd on gastropods under natural, but controlled conditions could be obtained.

Helisoma trivolvus was selected for our initial work because its natural habitat is similar to the littoral habitat created in our experimental system and they were easily collected in large numbers on the SRP. Also if H. trivolvus could be successfully transplanted into our system with good survival, sufficient data would be acquired to enhance our chances of success

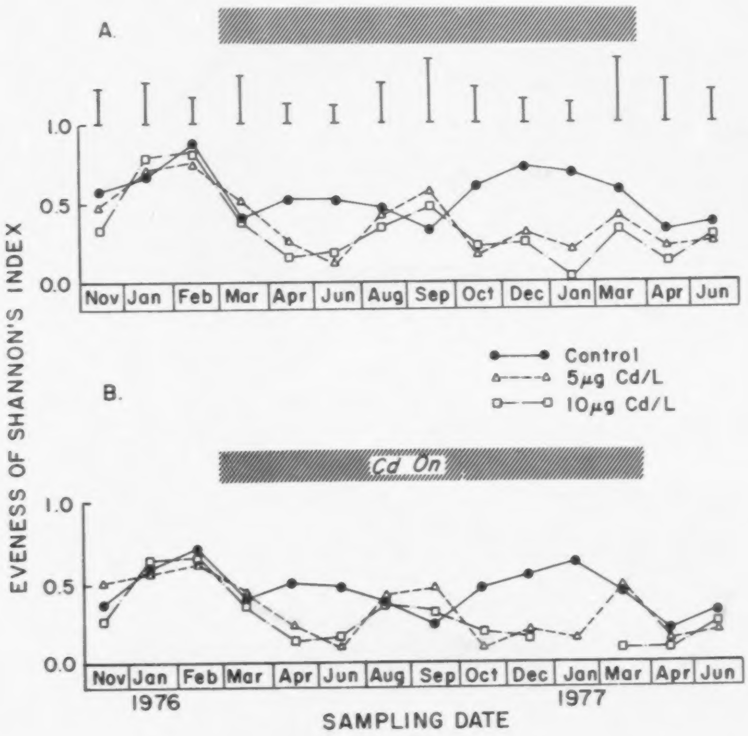


Figure 41. Evenness of Shannon's diversity index. A, means calculated across sampler by sampling period with two standard error confidence intervals indicated. B, calculated by summation.

with *C. lima*. *Camploma lima* being a prosobranch snail, is a gill breather which as a group are more limited to flowing or at least well oxygenated waters than are the pulmonates such as *H. trivolvus*. Therefore, if *H. trivolvus* would not survive our system successfully then the chances of *C lima* success were slim.

Six hundred *H. trivolvus* were collected by hand from Par Pond on the SRP, acclimated for two to four weeks, and 100 were transplanted into each channel. Transplanted organisms were marked with a dot of fingernail polish to distinguish them from offspring produced during the study. Initially, *H. trivolvus* appeared to adapt well to our experimental system. However, two weeks after their introduction, mass mortalities began occurring in all channels, prior to Cd input. This phenomena continued after Cd input began without alteration and by the end of three months no *H. trivolvus* or even relic shells could be found in any of the experimental channels. As a result of our experiences with *H. trivolvus* it was decided not to attempt to transplant *C. lima*.

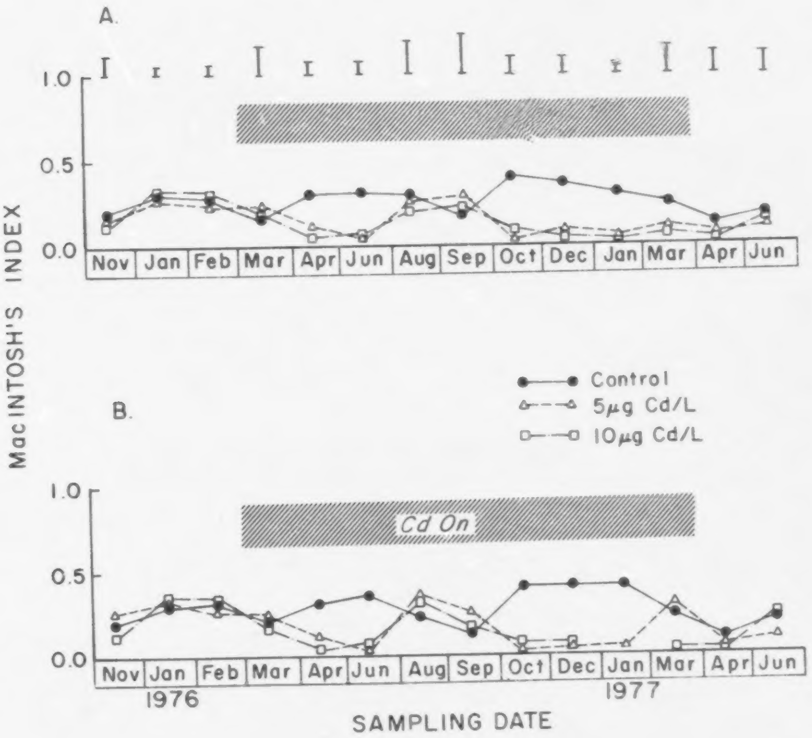


Figure 42. MacIntosh's diversity index. A, calculated across sampler by sampling period with two standard error confidence intervals indicated. B, calculated by summation.

The two pelecypods used in the study were Anodonta imbecilus (papershell clam) and Corbicula fluminea (Asian clam). Anodonta imbecilus was selected because it is common in the softwater ponds and reservoirs of the southeastern United States and because it could be collected readily. Corbicula fluminea was chosen because it is an ubiquitous nuisance species throughout the United States and is reported to be tolerant of environmental stresses, allowing colonization to occur nearly anywhere adults and/or larvae can migrate. All clams were collected by hand and acclimated for two to four weeks prior to being transplanted into the channels. Thirty two A. imbecilus of various sizes and 150 C. fluminea each of two size classes (1.7 cm and 2.6 cm shell length) were placed into the tail region of each channel. All organisms were placed directly into compartmentalized areas of channel sediment where they were allowed to move freely.

Anodonta imbecilus adapted well to our experimental situation, moving freely and filtering regularly. However, they did not survive due to heavy crayfish predation. The crayfish, Procamberus acutus acutus, which we had introduced into the channels, were observed crushing the paper thin outer

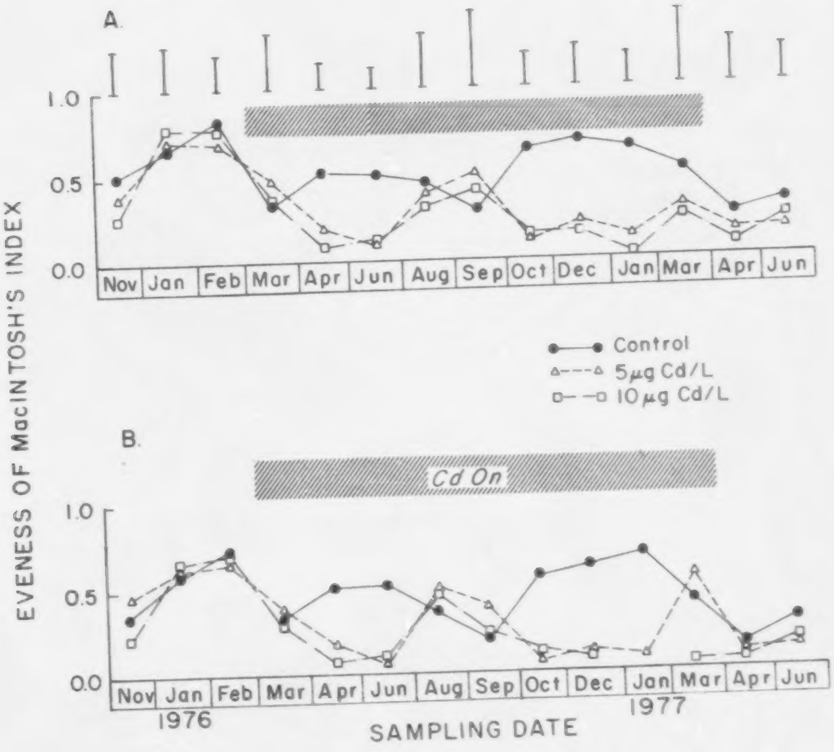


Figure 43. Evenness of MacIntosh's diversity index. A, calculated across sampler by sampling period with two standard errors confidence intervals indicated. B, calculated by summation.

The two pelecypods used in the study were *Anodonta* imbecilus (papershell clam) and *Corbicula fluminea* (Asian clam). *Anodonta imbecilus* was selected because it is common in the softwater ponds and reservoirs of the southeastern United States and because it could be collected readily. *Corbicula fluminea* was chosen because it is an ubiquitous nuisance species throughout the United States and is reported to be tolerant of environmental stresses, allowing colonization to occur nearly anywhere adults and/or larvae can migrate. All clams were collected by hand and acclimated for two to four weeks prior to being transplanted into the channels. Thirty two *A. imbecilus* of various sizes and 150 *C. fluminea* each of two size classes (1.7 cm and 2.6 cm shell length) were placed into the tail region of each channel. All organisms were placed directly into compartmentalized areas of channel sediment where they were allowed to move freely.

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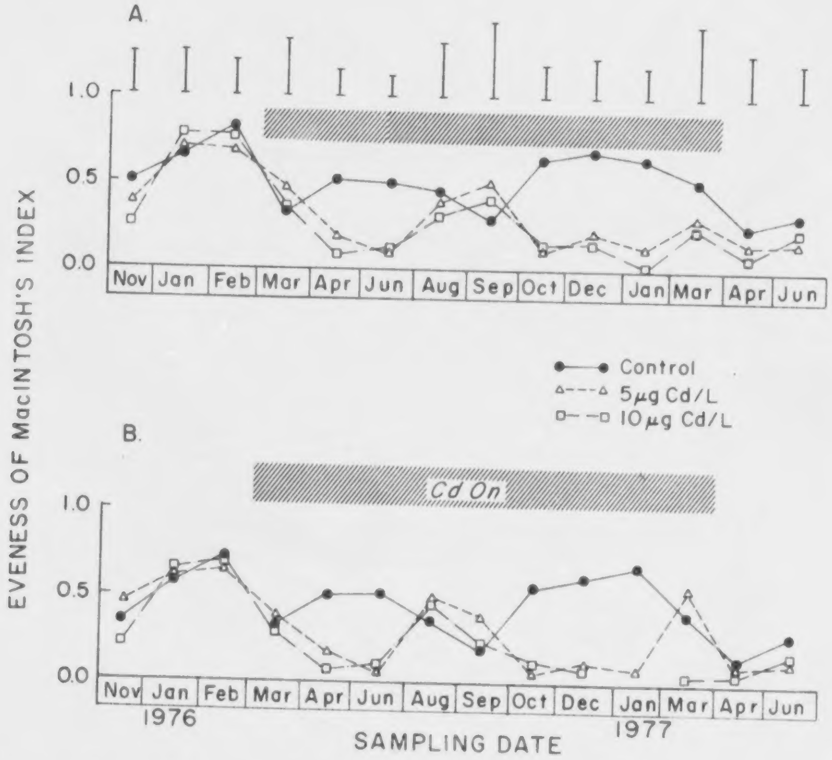


Figure 43. Evenness of MacIntosh's diversity index. A, calculated across sampler by sampling period with two standard errors confidence intervals indicated. B, calculated by summation.

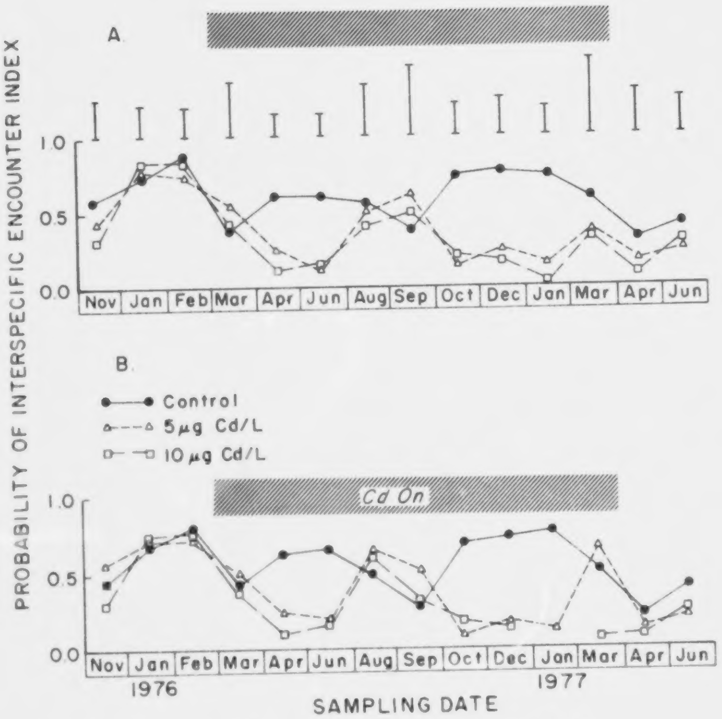


Figure 44. Probability of interspecific encounter diversity index. A, calculated across sampler by sampling period with two standard errors confidence intervals indicated. B, calculated by summation.

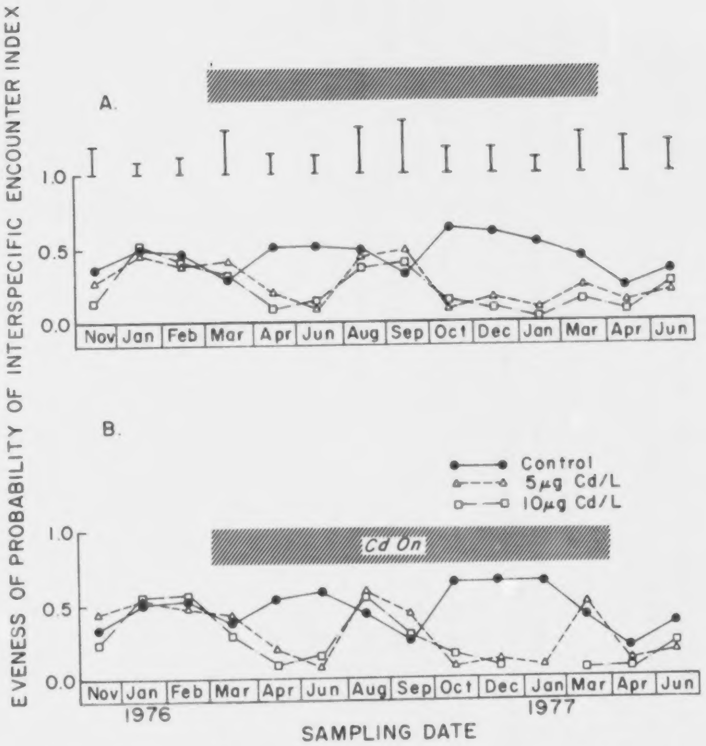


Figure 45. Evenness of probability of interspecific encounter diversity index. A, calculated across sampler by sampling period with two standard errors confidence intervals indicated. B, calculated by summation.

margins of the clam's shell, damaging the mantle flap and detaching portions of the mantle from the shell. Once damaged, A. imbecilus died rapidly and were eaten by the crayfish.

Corbicula fluminea reacted somewhat differently than did A imbecilus. They were unaffected by the crayfish, but never adapted to the system. They continued to show high mortality both before and after Cd exposure.

The abnormally high mortality of H. trivolvus and C. fluminea observed during our study was attributed to water quality. Water quality of our experimental system was initially thought to be adequate to sustain small populations of locally occurring molluscus species. As a result of the abnormal mortality rates observed early during the Cd study, however, a detailed review of all water chemistry parameters was conducted. From the results of

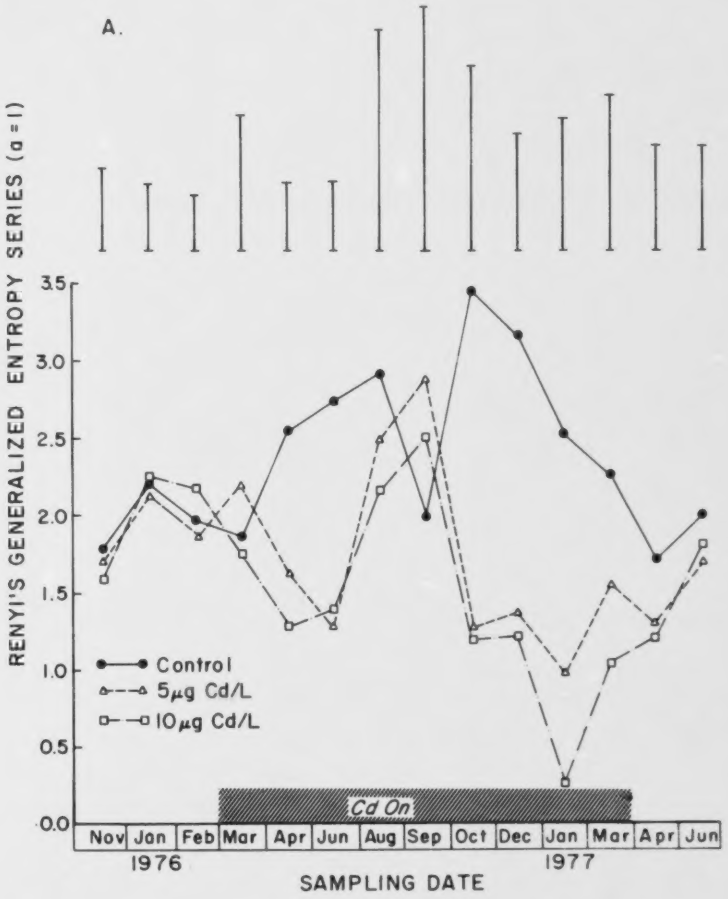


Figure 46. Renyi's generalized Entropy series (a = 1) calculated across sampler by sampling period, with two standard errors confidence intervals indicated.

this review and concurrent testing of all water quality parameters, it was determined that calcium levels in our system were not being maintained at levels previously believed to exist. Wetzel 1974, states that calcium has been implicated in numerous ways in the growth and population dynamics of freshwater flora and fauna. Wilbur and Yonge (1964) indicate that the most important chemical variable in determining the occurrance and distribution of molluscus in the environment is calcium. Although we could find no information about exact levels at which calcium determines the presence or absence of molluscs, we could relate such measures as hardness and alkalinity (as relative measures of total calcium) in our system to reported levels which have appeared to limit molluscus distribution. Mean hardness, calcium, and alkalinity values for our system were 29.0 mg/l, 10.8 mg.1, 9.9 mg.1, respectively. Harman (1969) has reported only a few molluscs surviving at levels around 21 ppm. While Harman (1970) and Pennak (1953) have both indicated that a total alkalinity of 15 mgl appears to be essential for the welfare of molluscan populations. Therefore, the results of other researchers suggests that our water quality is inappropriate to conduct studies involving molluscs (with the possible exception of some of the Anodontinae which are known to be softer water species). Even with the occurrence of abnormally high mortality and the potential for water quality effects, C.

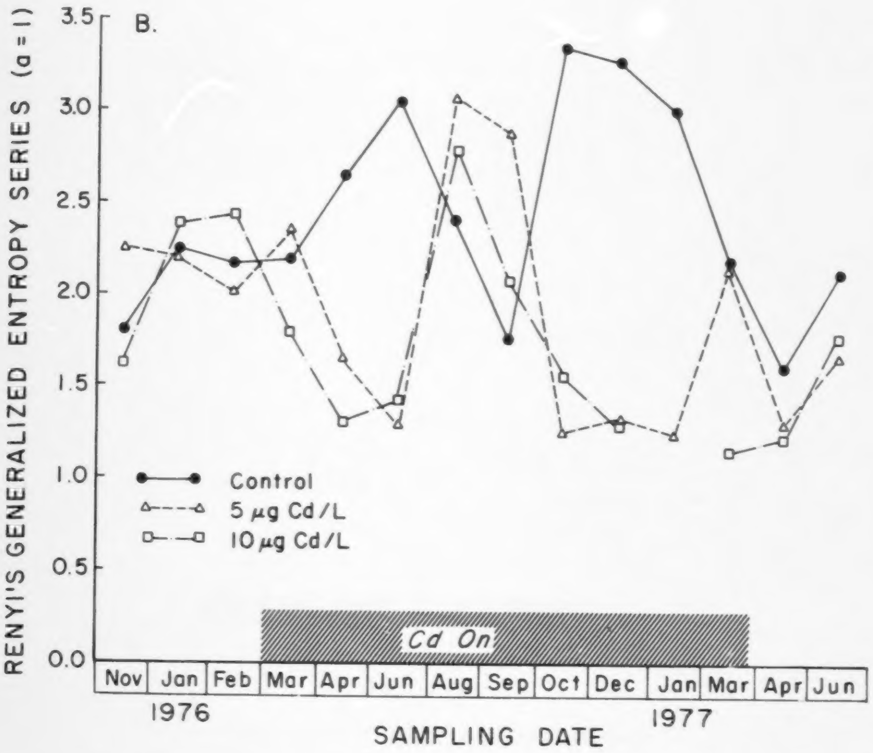


Figure 47. Renyi's generalized entropy series (a=1) calculated by summation.

fluminea populations displayed effects attributed to Cd exposure in both size classes utilized. The tendency for mortalities in treated and control organisms to diverge at approximately 13 days after Cd inputs began, resulted in significantly higher mortality levels for treated organisms than controls (Figures 50 and 51). Median survival times for large-size-class treated and control organisms were 20.8 and 29.2 days, respectfully. While small size class organisms possessed median survival times of 17.5 and 27.5 days for treated and control organisms. Therefore, results indicate that smaller organisms were more susceptible to Cd and that Cd is probably acting as an additive stressor in this case, causing treated organisms to die approximately 10 days before controls. Data also indicate that the total number of deaths occurring in either size class is not significantly affected by treatment.

A significant difference in mortality rates of small size class organisms at different treatment levels did occur during a 10 day interval beginning three days after Cd inputs started (Figure 50). Results indicate that during this interval higher mortalities occurred in 5 μg/l treated organism than in the 0 μg/l or 10 μg/l treated organisms. This observation may well

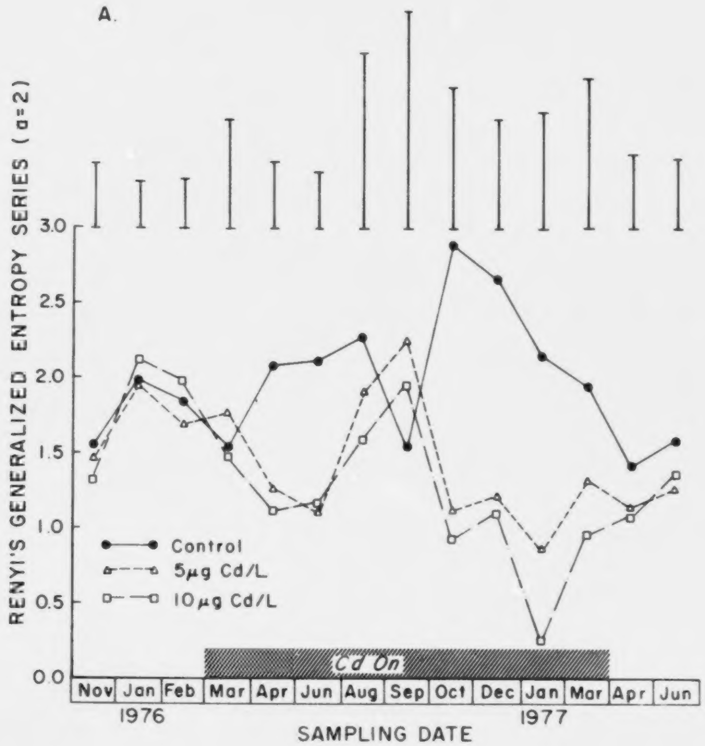


Figure 48. Renyi's generalized entropy series (a = z) calculated across sampler by sampling period, with two standard error confidence intervals indicated.

be explained by avoidance mechanism utilized by pelecypods where they just close up, stop filtration processes and essentially go into anaerobic metabolism maintaining only minimal bodily functions. This condition may be maintained until sensory receptors detect that the perturbation has passed or until metabolic waste products reach a potentially hazardous level and the organism has to start filtering again in order to eliminate waste. The result of this behavior is increased toxicant exposure at lower ambient toxicant levels. Harrison (personal communication) has observed similar results in low level metals toxicity work using pelecypods.

The unexpected mortality of molluscs in our system severely affected our efforts to collect Cd accumulation data. Therefore, no analyses are available for _H. trivolvus_ or _A. imbecilus_ Cd uptake and only limited data are available for _C. fluminea_ transplanted in the study (Table 17). Results indicate _C. fluminea_, like other mollusks, concentrate Cd. The levels accumulated appear to be in the same ratio as exposure levels; however, they are orders of magnitude greater. Another point of interest is that although Cd concentrations accumulated in 10 μg/l treated organisms are nearly double the levels found in 5 μg/l treated organisms, the additional Cd body burden did not significantly increase mortality.

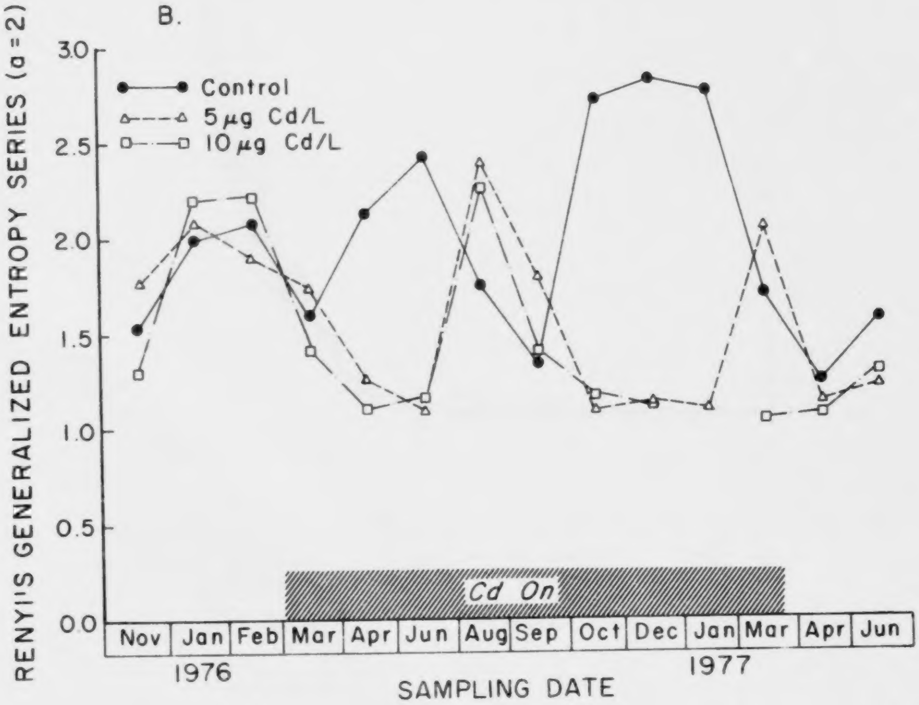


Figure 49. Renyi's generalized entropy series (a=z) calculated by summation.

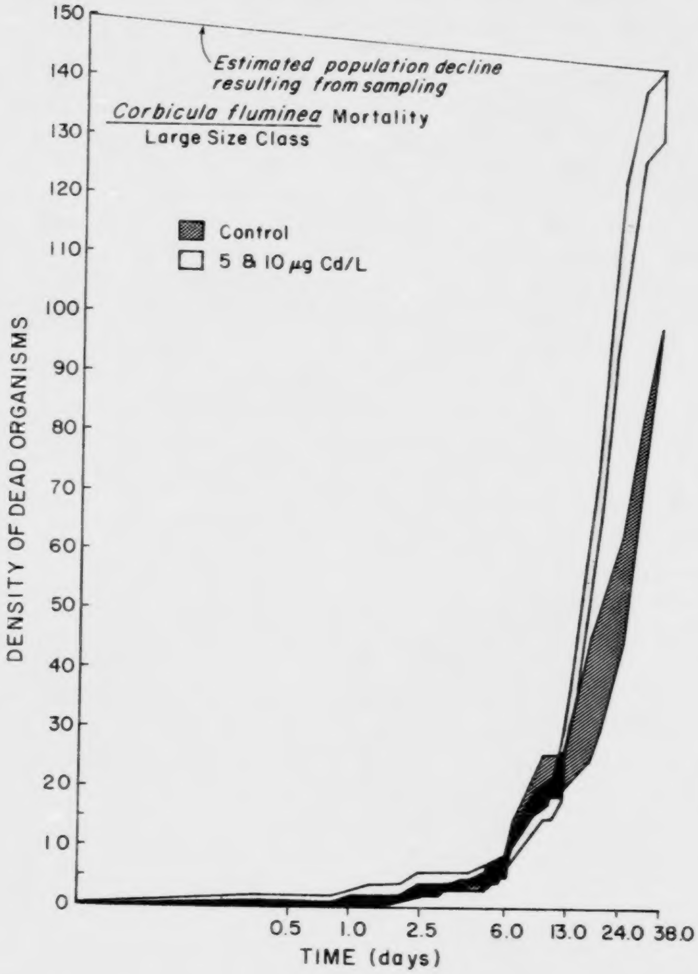


Figure 50. Mortality of large C. fluminea as a function of time.

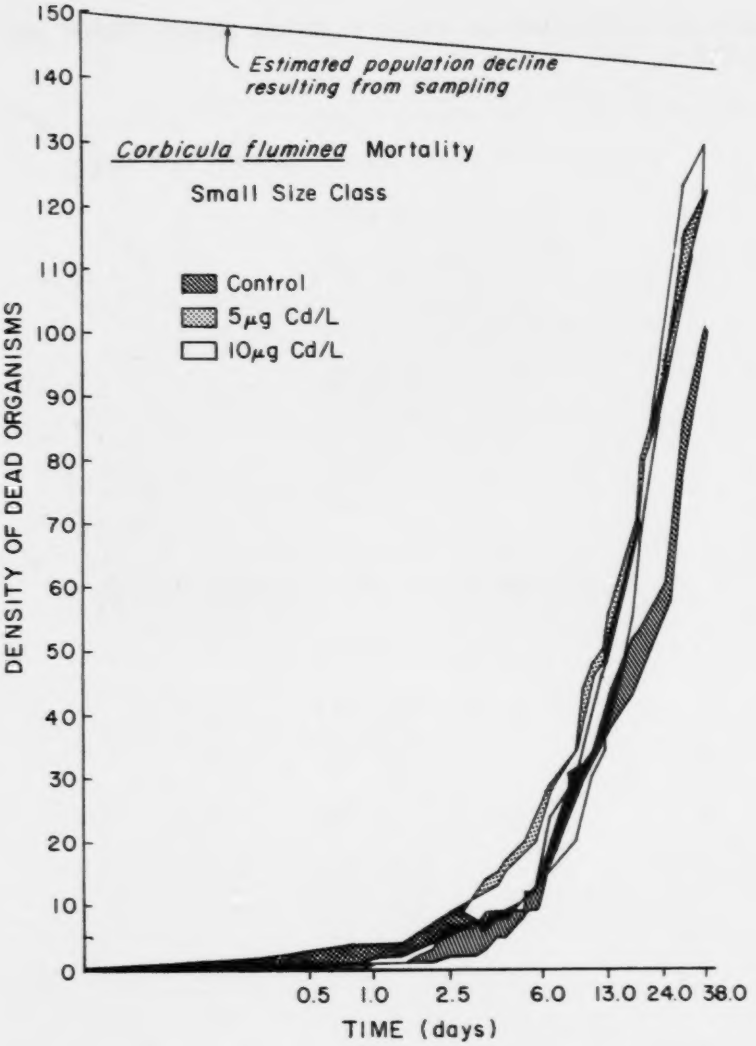


Figure 51. Mortality of small C. fluminea as a function of time.

TABLE 17. MEAN CD CONCENTRATION IN C. FLUMINEA WHOLE TISSUE EXPRESSED ON A DRY WEIGHT BASIS

	4/16/76	5/20/76
	Large Size Class (Shell Length 2.6 cm)	
Treatment	μg Cd/g	μg Cd/g
Background	2.29	----
0 ppb	3.60	5.24
5 ppb	43.76	----
10 ppb	62.36	62.88
	Small Size Class (Shell Length 1.7 cm)	
	4/16/76	5/20/76
Treatment	μg Cd/g	μg Cd/g
Background	3.83	----
0 ppb	6.06	8.37
5 ppb	36.10	54.31
10 ppb	77.02	123.66

SECTION X

FISH

INTRODUCTION

It has been determined that the occurrence of "itai itai" disease in Japan is correlated with fish which are able to concentrate Cd in their tissues (Piscator, 1974; Perry et al, 1976). Therefore, there is a decided need to assess the effects of Cd on fish populations since Cd in fish tissue can result in increased exposure to human. Measurements of acute toxicity in fish have documented species dependent toxic thresholds varying from less than 10 μg/l to greater than 55 μg/P (Kumada et al, 1973), from very low water concentrations (Cearly and Coleman, 1974; Eisler et al, 1972; Fowler, 1975; Kneip and Lauer, 1973).

Most previous studies of Cd toxicity to and uptake by fish have been conducted in short-term laboratory systems, or based on completely uncontrolled field sampling. Neither of these two types of information alone is useful in constructing predictive models of the environmental behavior of Cd or understanding of mechanisms of Cd accumulation. The microcosm system used here was a unique opportunity to observe accummulation of Cd by fish from continuous low water concentrations in a relatively natural complex habitat. The mechanisms of Cd accumulation by fish must be understood before valid models of Cd flux through fish populations or biotic communities can be formulated and elemental cycling patterns predicted (Hardisty et al., 1974; Miettinen, 1975). Fish can potentially accumulate metals from both ingested food items and directly from water. Kinkade and Erdman (1975) reported that aquatic organisms accumulated Cd faster from soft than hard water. This indicates that, in soft waters such as those of the southeastern United States, Cd may be rapidly transported into biotic components of aquatic communities. Two studies were conducted. One was to determine the relative importance of food and water as vectors of Cd uptake to the mosquitofish, a species ubiquitous in southeastern aquatic systems and a potentially important compartment in aquatic and terrestrial food webs. The other study was performed with bluegill and mosquitofish to determine long term Cd uptake rates.

The two species used were the common bluegill (Lepomis machrochirus) and the mosquitofish (Gambusia affinis). The bluegill is a carnivorous, warm water species, commonly found in lakes, ponds and streams having a moderate amount of vegetation, is frequently used as a bioassay organism and is a much sought after human food source. The mosquitofish, common in many southern lentic aquatic situations is a livebearer and a surface feeder (primarily mosquito larvae).

METHODS AND MATERIALS

Mosquitofish, (*Gambusia affinis*), were seined from Asphalt Pond, located on the SRP. Two hundred fish were randomly placed in each channel by 1 March 1976. During the first month fish were in the channels, there was high mortality, therefore dead fish were replaced until 18 March 1976, when Cd input was initiated. The pectoral fins of these fish were clipped into the ray so that they could be identified as initial fish, as opposed to fish born in the channels. From April through September 1976, four live mosquitofish were sampled monthly from each stream by dip net. The channels were checked daily and dead fish collected. Both dead and live sampled fish were wet weighed and frozen in plastic bags for future Cd analysis.

Bluegills (*Lepomis macrochirus*), were trapped from Rischer Pond located on the SRP. On 19 and 20 January 1976, 50 bluegills were placed in each head pool and tail pool. Dead bluegills were replaced until 18 March 1976 when Cd input was initiated. On 4 March 1976 all bluegills were removed by electrofishing and replaced with 20 fish from Rischer Pond 9 March, 1976. In addition, 10 bluegills trapped from Par Pond, located on the SRP were added to each tail pool. Beginning 20 April and lasting through September, 1976, a single bluegill was sampled from each pool monthly. Both live sampled and fish found dead were frozen in plastic bags for Cd analysis.

To determine the relative importance of Cd in food and water, a more controlled study was conducted. Mosquitofish were maintained in cages and fed either Cd contaminated food or clean food. Fish were acclimated for 72 hr in aquaria and separated by sex. Forty fish were randomly placed into each of four 30 x 51 x 11.5 cm (water depth) 1/32 inch (0.08 cm) mesh stainless steel wire cages at a female-male ratio of 4:1 resulting in a total of 160 experimental organisms. Fish were acclimated in flowing well water for 168 hr. Cages were suspended in PVC-lined concrete troughs receiving well water at a rate of 94.6 ℓ/min resulting in a water velocity of 1.3 cm/s. Two cages were suspended in flowing well water while two cages were suspended in flowing well water containing 10 μg Cd/ℓ. The Cd concentration was maintained by continuously metering in stock $CdCl_2$ with daily calibrated persistalic pumps. Stainless steel screens were used to decrease cage volume after each sampling to maintain constant fish to cage volume ratio throughout the experiment. Fish were fed *ad libitum* twice daily. Cages were cleaned daily, 30 minutes after feeding to remove excess food, feces and detritus.

Food consisted of Wardley's Basic Food Flakes (Wardley Products Co., Secaucus, New Jersey). Food flakes were blended to a fine powder and divided into two portions. One portion was spiked with $CdCl_2$, dried and reblended. The resulting powder had the same consistency as the unspiked food. Unspiked and spiked food had nominal Cd concentrations of 0.115 and 1.13 μg Cd/g dry weight, respectively. One-tenth gram of spiked food was placed in 1 ℓ of well water and allowed to stand for 5 minutes to determine the amount of Cd lost before it was consumed. Food was then centrifuged from solution, using a Sorvall SS-1 centrifuge equipped with a KBS continuous flow system (Sorvall, Norwalk, Connecticut). The recovered residue was dried, weighed analyzed for Cd.

The experimental design was a 2 (food) by 2 (water) by 4 (time) three way fully crossed design. Five fish were removed from each cage after 2, 4, 6 and 8 weeks for Cd analysis. Nominal Cd concentrations in the water were < 0.02 and 10 μg Cd/ℓ; while those in food were 0.1 and 1.0 μg Cd/g dry weight. Data analyses were conducted with an IBM 360 model 195 computer using the Statistical Analysis System (Service, 1972). Factorial effect means were computed directly (Cochran and Cox, 1971). Significance of factorial main effects was tested using 2-way analysis of variance (ANOVA) within time.

Comparisons of effect means within sampling were made using T-tests in the absence of significant interaction terms (week 2, 4, and 6) and are presented with 95% confidence intervals. Comparisons of simple effect means in the presence of a significant interaction term were made using Tukey's honestly significant difference test and 95% confidence intervals for each simple effect mean are reported (Kirk, 1968).

Fish were freeze-dried, weighed and wet ashed in fired porcelain crucibles using 2-4 ml redistilled HNO_3, depending on sample weight. Samples were heated to 70° C on a hot plate until NO_2 evolution was negligible. Samples were cooled, 1 mℓ H_2O_2 added and to 70° C until all NO_2 evolution ceased. Fish food was digested in a similar manner.

The samples were allowed to cool to room temperature, diluted to 25 ml with denionized HOH and stored in washed polyethylene bottles. Fish samples were analyzed, using a Perkin-Elmer model 306 atomic absorption spectrophotometer equipped with an HGA-2100 flameless atomizer and deuterium continuum background corrector. Standard additions were performed and no significant matrix interferences were found. (See Appendix I).

RESULTS AND DISCUSSION

Sampling fish was difficult because bluegills escaped from the pools into the channels. Also, additional fish added to compensate did not retain marks well and could not be identified from those that had been present for longer periods of time. Also, birds often ate or partly destroyed bluegills which died in the channels.

The mean Cd concentration in mosquitofish collected from Asphalt pond was 0.45 ± 0.16 μg Cd/g, dry weight (n = 6, ± 2 SE). The mean initial Cd concentrations in bluegills was 0.39 ± 0.19 μg Cd/g, dry weight (n = 10, 2 SE), which is similar to that reported for southeastern bluegills (Giesy and Wiener, 1977). Wet weight to dry weight ratios for mosquitofish and bluegills were 0.31 and 0.28 respectively. Mosquitofish rapidly accumulated Cd from both the 5 and 10 μg Cd/ℓ treatments (Fig. 54). Fish exposed to 10 μg Cd/ℓ exhibited a significantly higher rate of accumulation.

This result is similar to that observed by Merlini _et al_., (1973) for _Lepomis gibbosus_. The Cd concentration in _G. affinis_ tissue did not reach equilibrium in either Cd treatment during the 6 month exposure. Mortality of bluegill maintained in Cd treated channels was high and the bluegill population became extinct at one time, so accumulation results are not presented for this species.

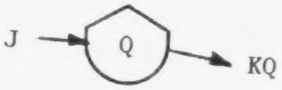
Although the mosquitofish had not reached an equilibrium value after 180 days of exposure, they did exhibit a leveling off trend (Fig. 52). For this reason, uptake rates for this population were calculated using the first 130 days of exposure. The rate of Cd accumulation by G. affinis on the first 130 days of exposure can be described by linear regression models (Table 18).

TABLE 18. LINEAR MODELS OF THE FORM y = mx + b of Cd UPTAKE BY G. AFFINIS

	Cd Exposure	Concentration
	5 μg/L	10 μg/L
Slope (m) $\left(\frac{\mu g\ Cd/g\ dry\ wt}{day}\right)$	0.14	0.23
SD of m	0.01	0.02
Intercept (μg Cd/g dry wt)	1.01	1.02
R	0.93	0.95
N	27.	19.

The results of this analysis indicate that the rate of accumulation of fish exposed to 10 μg Cd/ℓ was approximately twice that of fish exposed to 5 μg Cd/ℓ. There was less lag in Cd accumulation in fish exposed to 10 μg/ℓ than those exposed to 5 μg/ℓ.

The fact that organisms achieve different equilibrium Cd body burdens may be due to several mechanisms. A possible mechanism is a constant elimination rate (KQ) and donor controlled uptake rate (J) (equations 7 and 8)



(7)

Uptake rate can be described by equation

where: $$\frac{dQ}{dt} = J - KQ \qquad (8)$$

Q_o = steady state Cd concentration

T = time
K = constant
Steady state concentrations (Q_{ss}) can be calculated using equation ($Q_o = \frac{J}{K}$)

Cadmium accumulation by G. affinis residing in the channels receiving Cd were fitted to the Von Bertalanfly model (using the Gauss-Newton iterative least squares technique, (Barr *et al.*, 1978)) with derivatives of the form $\frac{dQt}{Co} = 1 - (e^{-KT})$ and $\frac{dQT}{K} = (C_o)(T)(e^{-KT})$. The predicted equilibrium concentrations (Q_o) are 35.7 and 61.0 μg Cd/g dry wt for fish exposed to 5 and 10 μg Cd/ℓ respectively (Table 19). The estimated uptake constants for fish exposed to 5 and 10 μg Cd/ℓ are 0.0058 and 0.0054, respectively. Uptake of Cd by fish exposed to 5 and 10 μg Cd/ℓ fit quite well by the Von Bertanfly model (Table 19).

A 3-way ANOVA was used as the preliminary test of significance of food and water sources of Cd in the cage study but the power of the test was much reduced due to the large differences in variances between treatments. Since the primary aspect of this study was the main factorial effects and interactions independent of time, differences in Cd body burdens were tested using

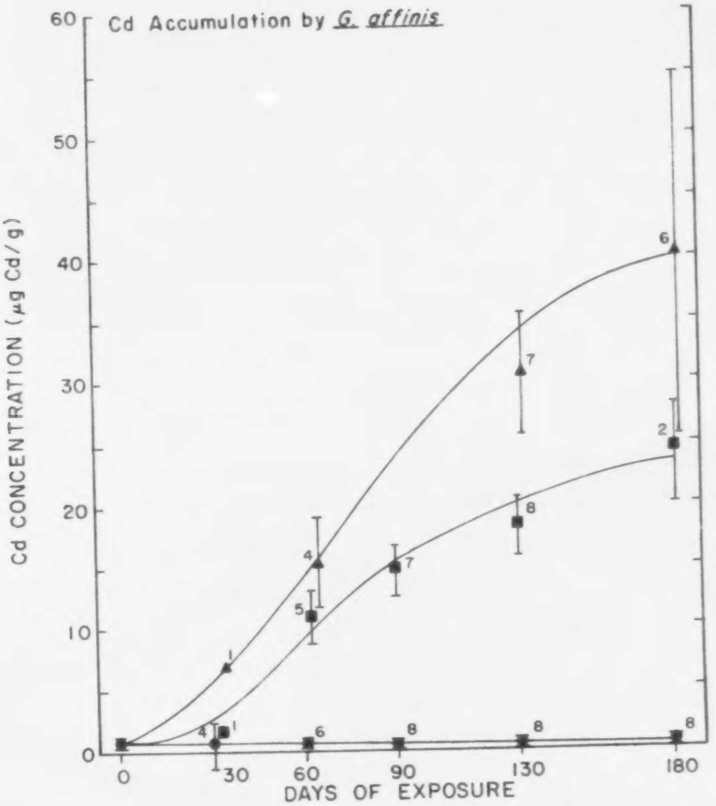


Figure 52. Cadmium accumulation by mosquitofish (G. affinis) n and 2 SE are indicated.

TABLE 19. NON LINEAR LEAST SQUARES FIT OF CD ACCUMULATION BY *G. AFFINIS*. DATA FIT TO $Q = Q_o (1 - e^{-KT})$ USING THE GAUSS-NEWTON ITERATIVE TECHNIQUE.

5 µg Cd/L

Source	df	Sum of Squares	Mean Square
Regression	2	9775.19	4887.59
Residual	33	526.20	15.94
Uncorrected total	35	2708.59	

Parameter	Estimate	Asymptotic Standard Error
Q_{ss}	35.78	10.89
K	0.0058	0.0027

10 µg Cd/ℓ

Source	df	Sum of Squares	Mean Square
Regression	2	17216.53	8608.26
Residual	23	1965.13	85.44
Uncorrected total	25	19181.66	

Parameter	Estimate	Asymptotic Standard Error
Q_{ss}	61.00	36.43
K	0.0054	0.005

$p < 0.001$

2-way ANOVAS within sampling period. While making the statistical tests within sampling time more powerful and facilitating the reporting of the results of the analysis, the ability to compute rigorous statistical tests across time was lost. Treatment effects on fish dry weight were tested using the 3-way ANOVA since the variances were more similar and the factorial effects were independent. For ease of reference, mean Cd concentrations with 95% confidence intervals are reported for each treatment combination at each sampling (Table 21) even though these values can be calculated from the factorial main effect means (Tables 20 and 22).

There was no significant increase in Cd levels in fish maintained in water containing less than 0.02 μg/ℓ Cd regardless of food ration (Tables 20, 21 and 22). Fish maintained in water containing 10 μg Cd/ℓ had significantly higher Cd concentrations than those maintained in low Cd water after 2, 4, 6 and 8 wk (Tables 20 and 22). Cadmium concentrations due to waterborne Cd plateaued between week 4 and 6 before sharply increasing between week 6 and 8.

Water was a highly significant source of Cd to mosquitofish throughout the experiment (Tables 20, 21 and 22) indicating Cd is taken up directly through the gills (Kumada _et al_., 1973). Hiyama and Makoto (1964) reported that the gills had the highest Cd concentration of any organ and suggested Cd transport across gill membranes as an uptake mechanism. Mummichog also accumulate Cd directly from seawater under continuous flow conditions (Eisler _et al_., 1972).

TABLE 20. FACTORIAL MAIN EFFECTS OF CD LEVELS IN FOOD AND WATER ON WHOLE BODY CONCENTRATIONS OF CD IN MOSQUITOFISH WITH 95% CONFIDENCE INTERVAL AND F-TEST (P), n = 5.

		Main Effects (μg Cd/g dry wt)			
Week	General Mean (M)	Water (W)	Food (F)	Water Food (WF)	95% CI
2	8.85	+15.1 (*)	+2.00 (NS)	+1.46 (NS)	±11.6
4	13.97	+25.5 (**)	-1.36 (NS)	-1.08 (NS)	± 7.5
6	16.07	+24.5 (**)	-2.4 (NS)	-2.4 (NS)	±10.2

*$p < 0.01$
**$p < 0.001$

TABLE 21. MEAN CD CONCENTRATION IN MOSQUITOFISH UNDER FOUR TREATMENT COMBINATIONS OVER TIME, C.I. = 95%, N = 5.

Week	Water	Response (µg Cd/g)*	
		Food	
		L	H
2	L	1.60 ± 1.48	1.07 ± 0.68
	H	14.67 ± 2.33	18.14 ± 17.07
4	L	1.48 ± 0.64	0.93 ± 0.17
	H	28.10 ± 10.6	25.38 ± 2.85
6	L	3.86 ± 2.58	3.82 ± 3.14
	H	30.72 ± 14.16	25.88 ± 2.86
8	L	0.41 ± 0.12	0.61 ± 0.22
	H	46.88 ± 12.57	71.49 ± 16.49

*dry weight

There was no significant difference between Cd concentrations in fish fed high Cd level food and those fed low Cd food at either the high or low water Cd concentration through week 6 (Tables 20 and 21). When 0.5 g of spiked fish food was soaked in 1 ℓ water, 0.42 g was recovered. The recovered food had a Cd concentration of 0.95 µg/g dry wt, or 84% of the added Cd. Feeding was generally complete in 5 min so fish were exposed to a considerable amount of Cd via the food pathway. Food was a significant source of Cd only after 8 weeks where the only significant interaction between food and water sources occurred (Table 22). The significant interaction between food and water Cd sources is indicative of non-additivity between these two factors. Consumption of Cd spiked food did not increase whole body Cd concentrations in fish maintained in low Cd water. The positive interaction term indicates that more Cd was accumulated than could be explained by either factor acting alone, which may have been due to physiological changes induced by the previously accumulated Cd. This significant interaction may indicate two uptake mechanisms which are integrated. Food may become an important uptake vector only after a threshold body burden is reached causing a de-

TABLE 22. SIMPLE EFFECTS AND INTERACTION TERM FOR WEEK 8 WHICH INCLUDES A SIGNIFICANT INTERACTION BETWEEN FOOD AND WATER WITH 95% CONFIDENCE INTERVAL AND F-TEST (*P*), n = 5.

Simple Main Effects		95% CI	F-test (*P*)
Food at low water	0.196	21.0	NS
Food at high water	24.6	21.0	*
Water at low food	46.5	21.0	***
Water at high food	70.9	21.0	***
Food x water interaction	12.2		*

*p > 0.05
***p > 0.0001

crease in the fishes ability to restrict Cd influx via gastro-intestinal assimilation.

There were no significant differences in Cd concentration due to size or sex in exposed or unexposed fish. The mean live weights and dry weights of test fish did not change during the course of the experiment and were not affected by any of the treatment combinations.

Fassett (1975) suggests that an organism will accumulate Cd as long as there is a continuous supply and therefore will not reach equilibrium. Investigations concerning organisms attaining equilibrium concentrations vary depending on the type of system and organism. In a static system, Kinkade and Erdman (1975) showed catfish and guppies to reach equilibrium in 7 days, perhaps due to Cd depletion. In a flowthrough system, Cearley and Colemen (1974) found that bluegills and bass reached equilibrium in 2 months, whereas rainbow trout, when exposed to 1.0 μg Cd/ℓ attained equilibrium in 10-20 weeks (Kumada *et al*, 1973). After three months exposure to 10 μg Cd/ℓ, mosquitofish had approximately 6 times more Cd in their tissues than did rainbow trout exposed to 10 μg Cd/ℓ for 10 weeks (Kumada *et al*., 1973). Hiyama and Makoto (1964) found fish came to equilibrium with Cd in solution in 15 days but did not indicate whether this was under static or continuous conditions. Sullivan *et al*., (1978) reported that fathead minnows came to equilibrium with Cd in both laboratory and field experiments within 20 days. Miettinen (1975) found that ^{109}Cd administered in the diet of rainbow trout (*Salmo gairdneri*) was rapidly eliminated with only 1% of the administered dose remaining in the body after 42 days. Cadmium accumulation by white catfish is greatest in the gastrointestinal tract with little Cd accumulated in skin and gills (Rowe and Massaro, 1974). Hardisty *et al.*, (1974) reported that Cd in the tissues of marine fishes is related to the number of crustaceans in the diet, indicating food as an important pathway of Cd uptake. When dace were fed Cd contaminated food the amount of Cd accumulated was increased over water exposure concentrations alone (Kumada *et al.*, 1973). Fishes eliminate Cd through the kidneys, and fish removed from Cd containing water are able to reduce their body burdens of Cd by excretion (Kumada *et al.*, 1973). Cadmium accumulation has been linked with renal hypertension (Schroeder, 1974). The rapid increase in Cd accumulation after an apparent equilibrium in this study may be due to renal failure with a subsequent inability to excrete Cd. Another mechanism which may be responsible for the rapid increase in uptake after 6 weeks accumulation is induction of metalbothionein, a metal binding protein which prevents Cd binding to sulfhydryl containing enzymes (Fassett, 1974). Small doses of Cd are able to induce protection against subsequent massive doses (Fassett, 1974). Cearley and Coleman (1974) suggested a mechanism of elimination which is triggered after threshold concentrations are reached in excretory tissues such as kidney. In contrast, Eisler (1972) suggested that Cd does not accumulate in fish because it is actively excreted.

The relative importance of water and food as sources of Cd to fish may be dependent on many factors such as food quality, relative Cd concentrations in food and water, form of Cd in water and species of fish. This experiment was conducted under strictly controlled conditions to minimize variability. For the species studied, direct uptake from water is the more important vector of accumulation. Future investigations should involve effects of physical-chemical water parameters and use physiologically labeled food sources such as prey items. Comparisons of uptake of several essential and nonessential elements by a number of aquatic organisms are needed before comprehensive models of cycling and fluxing processes can be described and predictive models constructed.

Water quality is important in determining the availability of Cd to biota (Giesy *et al.*, 1977). Wiener and Giesy (1978) found fish residing in soft waters, such as those used in this study and common to many areas of the eastern United States, have higher concentration ratios for Cd then fish residing in harder waters. Since our research indicated that water was the primary source of Cd to *G. affinis*, concentration factors (equation 9) are an appropriate method of comparing relative availability between aquatic situations (Jinks and Eisenbud, 1972).

$$CF = \frac{C_o}{C_w} \qquad (9)$$

where:

C_o = Cd concentration in the fish, µg/g dry weight.
C_w = Cd concentration in the water, µg/P.
C_F = concentration factor.

For these comparisons of relative availability to be valid, the assumption of equilibrium conditions must be met. While this condition is not strictly met after 180 days of Cd exposure, Cd concentrations in the organisms seemed to be approaching an equilibrium. The concentration factors after 180 days exposure were 4.9 and 3.8 for the 5 and 10 μg Cd/ℓ treatments, respectively. From our data it is not clear whether the final equilibrium concentrations in fish exposed to 5 and 10 μg Cd/ℓ would be significantly different. There is no significant difference between the Cd concentration of fish exposed to 5 or 10 μg Cd/ℓ after 180 days, but this is due to the great variability of these data. Hamelink (1976) reports that the variability about the mean accumulation increases with time due to the inherant property of a population of animals expressing their individuality. Also the uptake and elimination processes involved tend to produce log-normal distributions of non-essential elements in aquatic organisms, causing an over estimate of population variability when represented as a mean and standard error (Giesy and Wiener, 1977). If in fact the final equilibrium Cd concentrations of fish populations exposed to similar Cd concentrations under different conditions are similar, concentration factors will not be useful in assessing relative availabilities between various systems.

Total metal concentrations may be the same and under different environmental conditions exhibit different availabilities because of differences in the actual concentrations of available metal. However, this was not the case in the system studied here where Cd was present in the same form in the channels receiving both 5 and 10 μg Cd/ℓ. Thus to assess relative availabilities of metals from different environments, the total concentrations in each environment must be equal. Comparisons of concentration factors calculated for fish studied in the channels to literature values would therefore be inappropriate.

Although this study was not designed as a toxicity bioassay and complete recovery of dead organisms was not assured, some information on chronic toxicity in a complex situation of exposure via both food and direct exposure in the water was gleaned. Mortality may be due to both direct toxicity and secondary effects of Cd exposure to other components of the system. There was little difference between mortality in control channels and those receiving 5 μg Cd/ℓ for either bluegill or mosquitofish, however, mortality in the channel receiving 10 μg Cd/ℓ was approximately twice that of the other two treatments (Table 23).

The bluegills initially placed in the channels were all dead within a few weeks when exposed to Cd. These animals had been exposed to multiple stressors. The fish had been starved to maintain a small size and were transported to the channels on a warm day. The second attempted stocking of bluegills captured in Par Pond on the SRP also exhibited high mortality when exposed to Cd in the channels. Necropsies of dead fish revealed these animals were highly parasitized with metacercaris of Diplostomulum scheuringi (Trematoda). When unparasitized, unstarved fish were collected from Rischer Pond, the initial mortality was much less. While this is not a rigorous test of these effects, it does indicate that a number of environmental and physiological parameters are important in determining Cd toxicity.

TABLE 23. BLUEGILL AND MOSQUITOFISH MORTALITY BETWEEN MARCH AND JUNE 1976.

Treatment µg Cd/ℓ	Bluegill (%)	Mosquitofish (%)
0	17	21
5	29	23
10	53	55

Ball (1967) found acute mortality of rainbow trout at 10 µg Cd/ℓ. He also, however, found 96-hr LC_{50} values of 1.0 µg Cd/ℓ for steelhead strout. Giesy _et al._, (1977) found LC_{50} of Cd to mosquitofish in the well water used in the artificial streams to be 0.9 and 2.2 at 30 and 28° C respectively. When comparing literature concerning metals toxicity in fish, it must be noted that Cd toxicity in fish will vary depending on water hardness, pH, alkalinity, temperature, dissolved oxygen and species (Giesy _et al_, 1977).

SECTION XI

LEAF DECOMPOSITION

INTRODUCTION

Prior to human perturbation, most streams and rivers were densely covered with vegetation. Shielding from direct sunlight and the structure of stream channels fostered the development of a heterotrophic based system. The dominant energy source of small woodland streams is allochthanous input (Petersen and Cummins, 1974). Only a small portion of the energy contained in leaf material is directly available to aquatic animals (Barlocher and Kendrick, 1974). The animal and microbial components of the streams community have evolved to process these inputs, with the animal community relying on micro-organisms to degrade recalcitrant plant substances such as lignin and cellulose. The microbial proteins, fats and carbohydrates are then readily available to animals which feed on them (Hargrave, 1970). Many stream dwelling invertebrates prefer to eat partly decomposed, or conditioned, rather than freshly fallen leaves (Kaushik and Hynes, 1971; Cummins, 1974) and may feed on the leaves to acquire highly nutritious fungal cells (Barlocher and Kendrick, 1973). Because of the importance of fungi and bacteria as intermediaries in leaf litter processing, their inhibition in streams would mean a drastic change in community structure and decreased secondary productivity. While the toxic and inhibitory properties of heavy metals to aquatic microbes have been studied, little is known about the effects of low levels of these toxicants on the colonization and leaf litter decomposition by microbial communities.

This study was part of a program designed to determine the biological effects of low levels of Cd (drinking water standards and below). Since leaf litter processing is important in lotic aquatic systems, this function was chosen as a critical function to be protected to maintain ecosystem integrity.

METHODS AND MATERIALS

Cadmium effects on the heterotrophic community were studied using leaf litter decomposition packs. Fresh leaf material was placed in 0.3 cm mesh, 15.2 cm square stainless steel envelopes (Fig. 53-54). These envelopes were tied at the top and sides such that 1.0 cm openings remained on each side. Leaf material was placed into each envelope in the order given in Table 24. Two each of Type I and Type II (Table 24) envelopes were suspended 10 cm above the bottom in each of the tail pools.

Leaf material was incubated in the tail pools for 28 wk between 28 April and 22 November 1976. Leaf material was removed and examined for macroinvertebrates. Total dry weight biomass was determined after drying at 85° C for 96 hr. Multiple undried samples of each leaf type were fixed in 2% Glutaraldehyde-0.1 $\underline{M}$ cacodylate buffer. Leaf samples were dehydrated by serially washing 15 min in 70%, 85%, 95% and 100% (twice) ethyl alcohol. Dehydrated material was critical point dried and mounted on aluminum stubs and gold coated for scanning electron microscopy. Each species was examined for fungal and bacterial colonization and permanent records made.

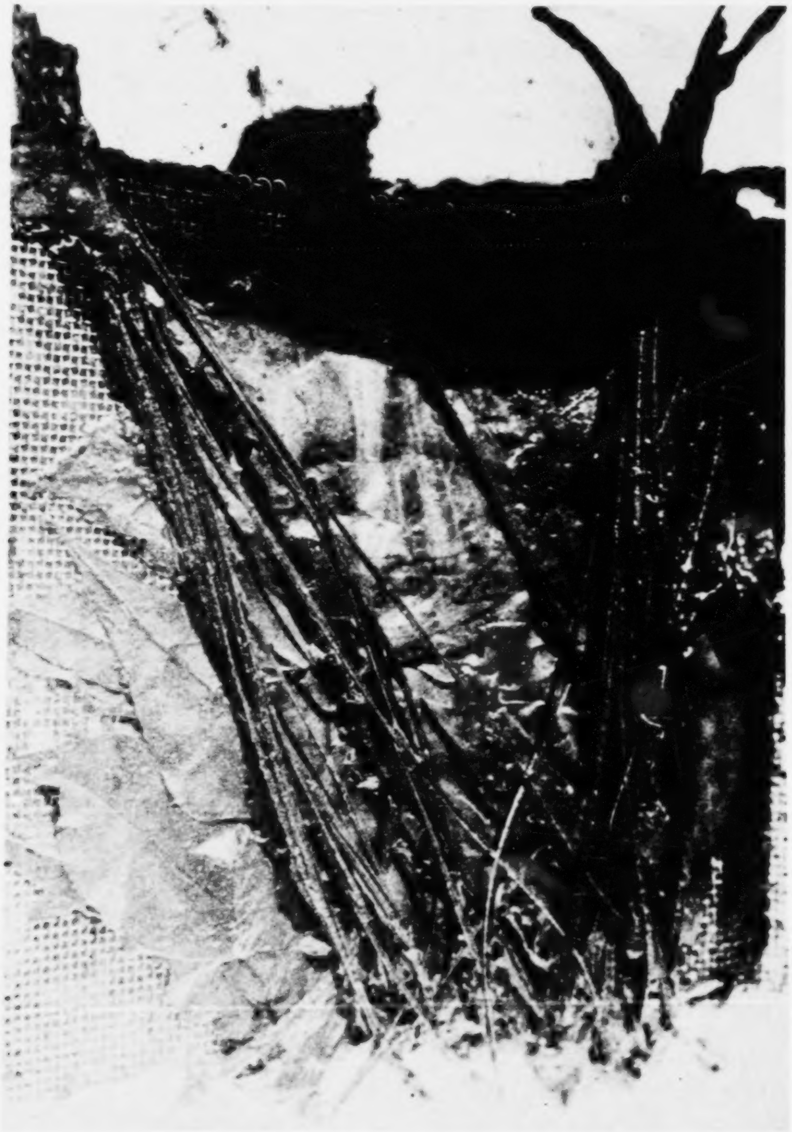


Figure 53. Leaf litter pack, Type I.

Leaf material was wet ashed using the methods reported for aquatic macrophytes (this report).

The experimental design was a randomized nested design with two treatments (5 and 10 µg/l Cd) and a control. There were two replicates of each treatment channel with two replicates of each leaf pack type in each channel resulting in four replicates of each leaf pack type per treatment. Results were analyzed by standard Analysis of Variance Techniques and significance of differences between means tested, using Tukeys'-w procedure (Steel and Torrie, 1960).

Figure 54. Leaf litter pack, Type II.

TABLE 24. INITIAL LEAF MATERIAL IN LEAF LITTER PACKS.

Species	Wet Weight Added To Each Envelope (g)
TYPE I	
Pinus taeda L.	5.0
Sassafras albidum (Nutt.) Nees.	3.0
Quercus nigra L.	3.0
Quercus laurifolia Michx.	2.0
Prunus americana Marsh.	2.0
Acer rubrum L.	2.0
TOTAL	17.0
TYPE II	
Acer rubrum L.	3.0
Quercus nigra L.	3.0
Prunus americana Marsh.	2.0
TOTAL	8.0

RESULTS AND DISCUSSION

Exposure to both 5 and 10 µg/l Cd significantly reduced leaf decomposition of Type I and II leaf packs (Table 25). There was no significant difference in leaf decomposition between Cd-treated channels for either litter pack type. The ratio between initial live weights and final dry weights of Type I and II leaf packs were 7.4 and 7.3, respectively.

Visual inspection of leaf material removed from the leaf packs, after 28 wk incubation revealed that leaves in 5 and 10 µg/l Cd had deteriorated much less than those in control water. Leaf material in control packs was brown in color and many of the leaves had only veins and petioles remaining. Leaves in the Cd treatments were green and completely intact. Microscopic examination revealed the intact structure of leaf surfaces, including leaf hairs and stomates. Within the controls, the order of resistance to decomposition, from least to greatest, was: S. albidum, P. americanum, A. rubrum, Q. laurifolia, Q. nigra and P. taeda. Although overall decomposition was reduced by the presence of Cd, S. albidum and P. americana were the most succeptible to decomposition, in the presence of Cd.

TABLE 25. EFFECT OF CD ON FINAL BIOMASS OF LEAF MATERIAL IN LEAF LITTER PACKS EXPOSED FOR 28 WK. ($\bar{X} \pm 2$ SD.)

Treatment	Dry Weight (g)	
	Type I	Type II
CONTROL	2.3 ± 0.18	1.1 ± 0.08
5 μg/L Cd	4.0 ± 0.06[a]	1.7 ± 0.19[b]
10 μg/L Cd	4.0 ± 0.73[a]	1.7 ± 0.11[b]

[a,b] not significantly different from one another, $n = 4$, $\alpha = 0.05$.

Few macroinvertebrates were found in the leaf packs. Two species of Odonata, Erythrodiplax minuscula Rambur and Ishnura sp., on species of snail, Limnea sp. and one species of flatworm were recovered from the leaf packs suspended in control channels. The only macroinvertebrate recovered from leaf packs incubated in treatment channels were flatworms.

Both 5 and 10 μg/l Cd inhibited microbial colonization of leaf surfaces (Figs. 55-58). Examination of leaf surfaces, using scanning electron microscopy (SEM) revealed the surfaces of leaves which had been suspended in treatment channels were almost devoid of microbial colonization, while the surfaces of leaves from control channels were well colonized. There were no apparent differences in colonization of the upper and lower leaf surfaces or position along the axes of pine needles.

Relatively little Cd was accumulated by leaf material suspended in the channels (Table 26). Uptake by leaf material was directly proportional to Cd concentration in the water.

When assessing the impact of a toxicant on an ecosystem, effects on the most susceptible component of that system should be determined. While particular components may not be of primary economic or aesthetic interest, they may be directly related to the overall desirability or productivity of a stable ecosystem. Such is the case of the aquatic microflora responsible for leaf litter decomposition in streams.

Many microorganisms are adapted to high Cd concentrations (Chopra, 1971; Doyle *et al*., 1975). A variety of fungi and bacteria have been shown to be tolerant to high concentrations of heavy metals, relative to concentrations which are toxic to other organisms (Asworth and Amin, 1964; Ashida, 1965). Cadmium presumably acts by inhibiting oxygen uptake. Sulfhydryl bonds, such as those in cysteine protect cells from Cd toxicity by binding Cd and preventing it from affecting enzyme systems (Tynelka and Zylinska, 1974). Cadmium does not affect *Escherichia coli* metabolism of ^{14}C- glucose until a Cd concentration of 6 mg/l is reached (Zwarun, 1973) and 10 μg/l Cd had no effect on the viability of a natural population of heterotrophic bacteria (Albright *et al*., 1972). Thormann (1975) found that the most sensitive estuarine bacteria were inhibited by 100 ppm Cd while the less sensitive species were able to grow in 400 mg/l Cd. Heavy metal resistant actinomycetes and bacteria have been isolated from soil near a zinc smelter which were capable of at least 50% of normal growth at 700 μm Zn (Jordan and Lechevaler, 1975).

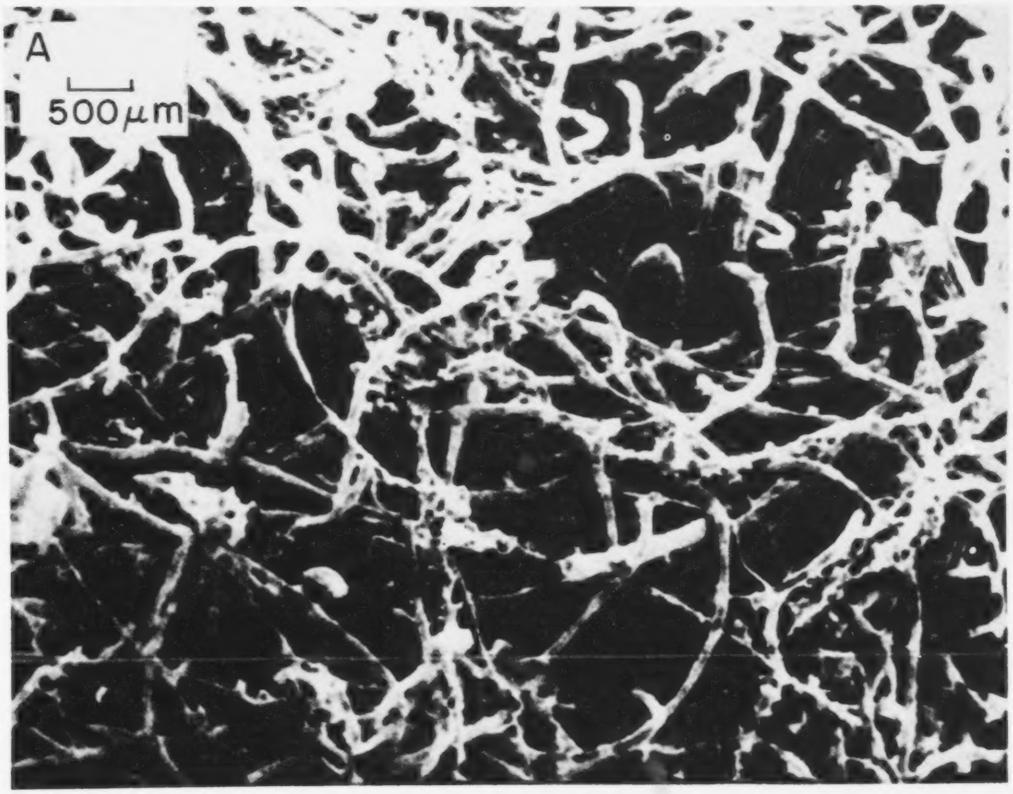


Figure. 55. Electron photomicrograph of the effect of Cd on microbial colonization of *P. taeda*. A. Control.

Leaf surfaces are rapidly colonized by fungi and bacteria under natural conditions (Iversen, 1973). Beech leaves, for instance, lost 90% of their weight during one year (Iversen, 1973). The results of our study indicate that low Cd concentrations can inhibit the functioning of decomposing microorganisms. Heavy metals such as copper, zinc and cadmium inhibit fungal spore germination (Ruhling and Tyler, 1973). Metals from a smelter have been found to disrupt microbial processes in terrestrial ecosystems and depress leaf litter decomposition (Auerbach et al., 1976), while metals such as Cd may affect the fungi colonizing the phylloplane of leaf surfaces (Gingell et al., 1976).

Natural microbial communities are more complex than the pure cultures often used to assess toxic effects of metals in laboratory studies (Albright et al., 1972). Assessment of toxic and inhibiting effects of low levels of heavy metals should be conducted in more complex situations than pure cultures, and substrates. Ramamoorthy and Kushner (1975) suggested that many synthetic media may complex heavy metals which may result in an underestimate of metal toxicity or inhibition which may occur under natural conditions.

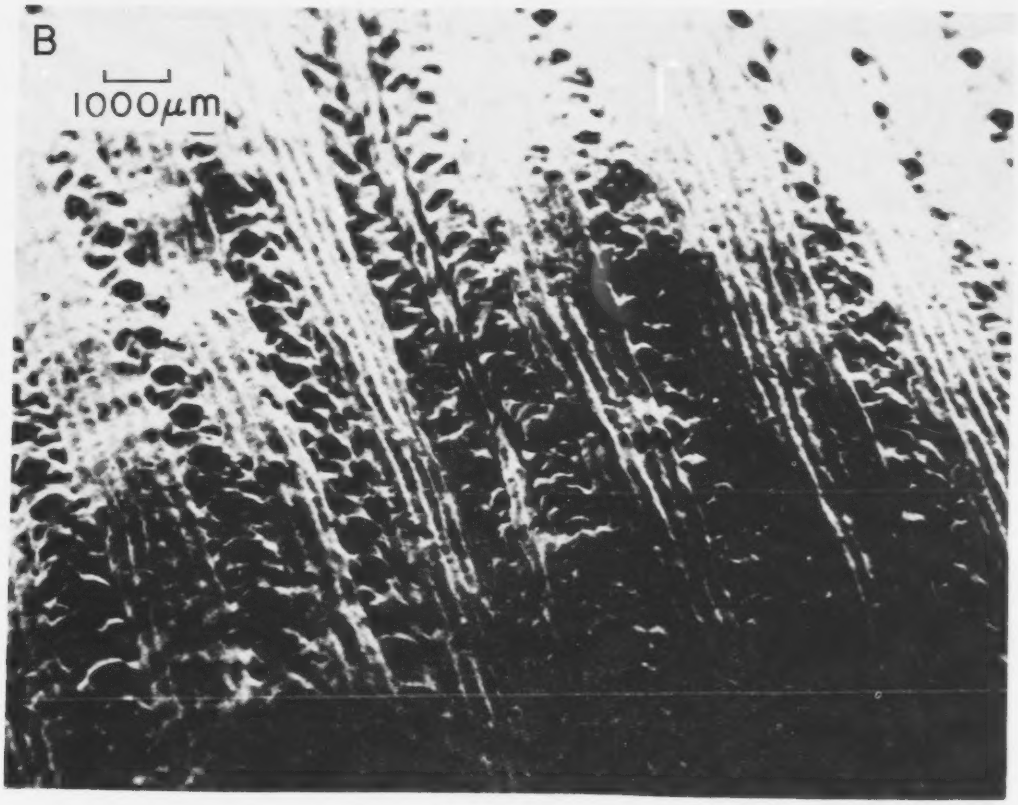


Figure 56. Electron photomicrograph of the effect of Cd on microbial colonization of P. taeda. B. 10 μg Cd/l.

TABLE 26. CADMIUM CONCENTRATION IN LEAF LITTER MATERIAL EXPOSED FOR 28 WK. ($\bar{X} \pm 2$ SD).

Treatment	Cd Concentration (µg/g dry weight) Type I	Type II
Control	2.8 ± 0.04	1.9 ± 0.01
5 µg/L Cd	8.5 ± 2.2	12.2 ± 3.4
10 µg/L Cd	18.4 ± 4.5	23.3 ± 7.8

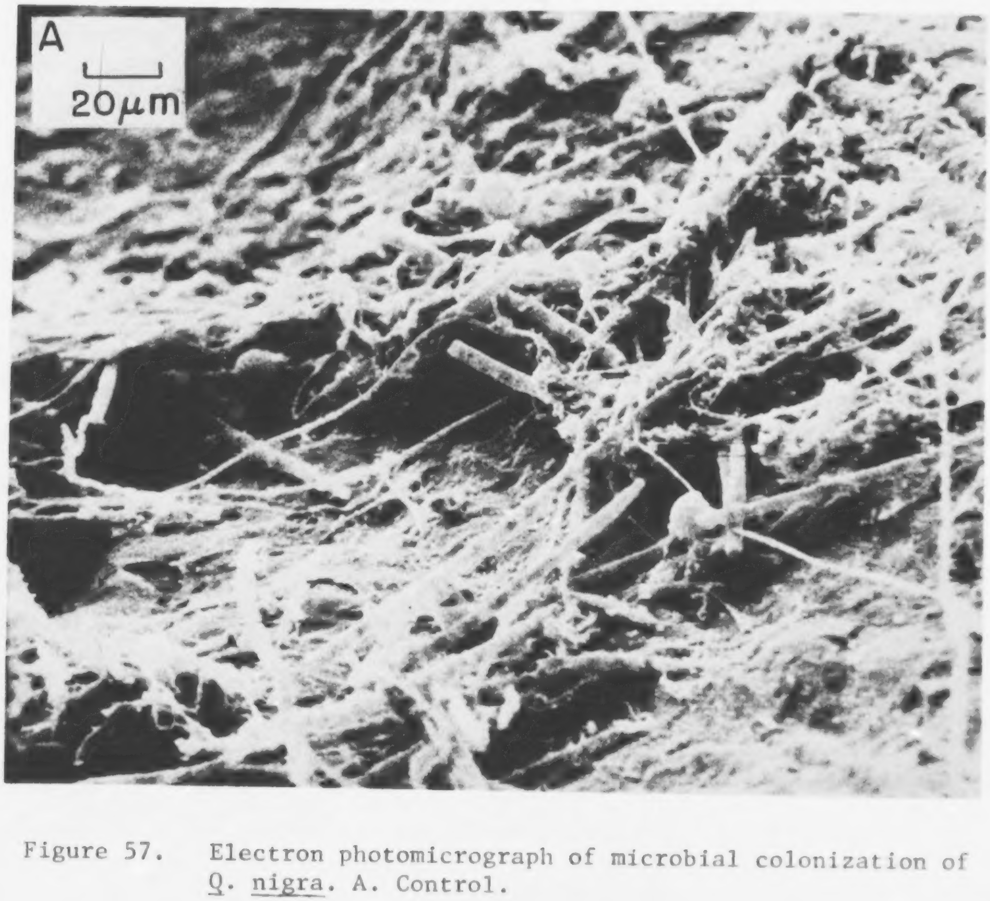


Figure 57. Electron photomicrograph of microbial colonization of *Q. nigra*. A. Control.

Batch pure culture bioassays do not represent the complex colonization procedure which may be the critical stage in microbial decomposition of leaf material under natural conditions. Bioassays, to determine toxic or inhibitory effects of compounds on processes as complex as microbial colonization and decomposition of leaf material must be conducted under conditions which account for the complete colonization process, species interactions and be of sufficient duration to allow for an organismal adaptation to occur.

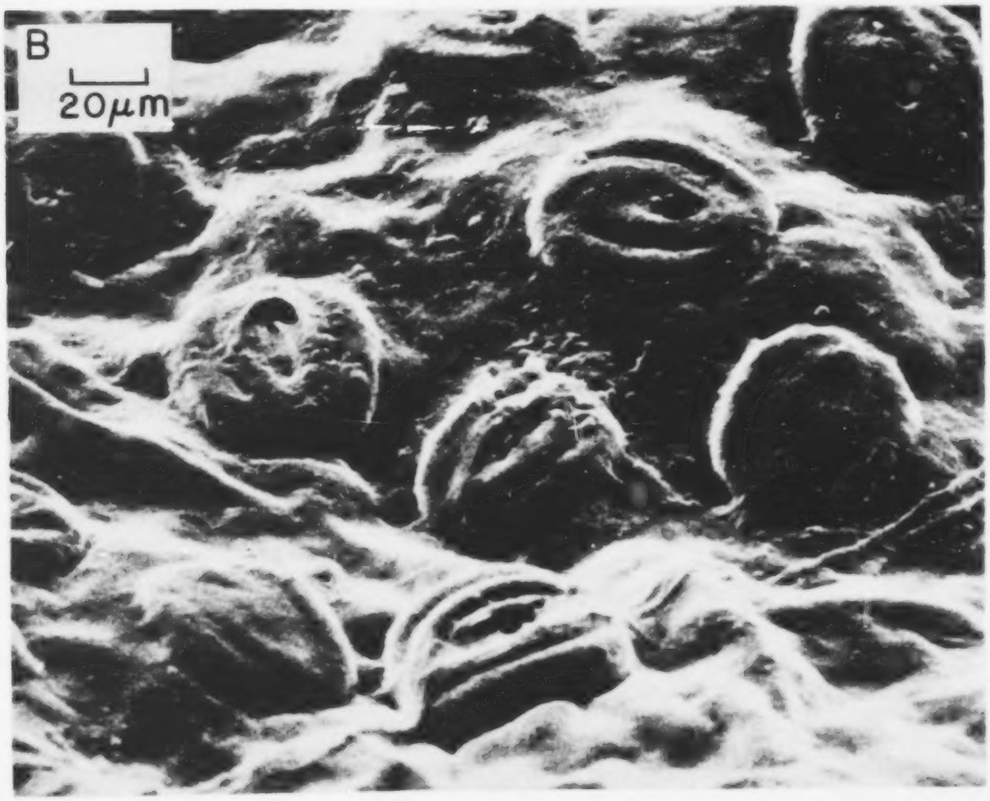


Figure 58. Electron photomicrograph of microbial colonization of Q. nigra. B. 5 μg Cd/1.

SECTION XII

SYSTEM RESPONSES

INTRODUCTION

Even a comprehensive knowledge of the biology of individual species does not provide enough information to accurately predict the complex interactions of communities. Maki and Johnson (1977a; 1977b) suggested the ratio of primary production to respiration (P:R) as a sensitive measure of environmental stress. Parameters which reflect structural and functional attributes of entire integrated systems are required. Some organisms may be more sensitive than others and some more important to overall system functioning than others. There are often functionally analogous species which may be interchanged with little effect on overall system functioning. Thus, rational assessment of impacts of potential environmental perturbations must be made in the context of what effects they will have on the entire community, and not what effect they will have on individual taxa.

The information presented to this point has been largely static, describing the condition of state variables of community and population structure at various times throughout the study. This section will report the effect of Cd on the dynamic ecosystem functioning measured by autotrophic production, system metabolism and system export. The last two parameters serve as integrators of the system's overall response to Cd and therefore may be most suitable in application of data from this study to other aquatic systems. An important property of natural flowing water systems is the export of organic material to downstream systems (Odum, 1957a). Also of importance is the retention and movement of a toxin in dissolved or particulate forms to downstream communities. Therefore, an effort was made to quantify the particulate organic matter and associated Cd leaving the streams in the effluent water. Systems level measures may prove to be more efficient and economical for assessing gross pollutional effects on aquatic communities as functioning natural units.

METHODS AND MATERIALS

Measurements of total community primary production and respiration were made on 30 June 1976, 28 July 1976, 23 September 1976, 20 October 1976, 24 November 1976, 9 February 1977, with a 24-hour upstream-downstream oxygen diurnal analysis (Odum, 1956). Water samples were removed from the streams by siphon at two hour intervals and dissolved oxygen content determined using a YSI Model 54 dissolved oxygen meter calibrated using the azide modification of the Winkler method (APHA, 1975).

In the spring of 1977 a semi-automatic method of collecting diurnal oxygen diurnal data was put into service. This system utilized 12 solenoid valves (one at the head and one at the tail of each channel), two YSI oxygen probes and meters, two timer boxes, and a chart recorder with another timer attached. At each end all six gravity-fed lines passed through solenoid valves into a single common line feeding the water over the end of the probe. Dissolved oxygen was monitored for ten minutes each hour. Signals from the corresponding meters were fed into a timer that switched input to the recorder at five minute intervals. In this manner, five minute recordings of dissolved oxygen concentrations at each location were recorded for each hour during day and night. Probes were calibrated several times during each 24 hour period.

The O_2 concentration for the head station was subtracted from the O_2 concentration in the same water mass at the tail station to determine oxygen changes. These values were corrected for diffusion by calculating percent saturation and using equation (10).

$$D = kS \qquad (10)$$

where:

D = diffusion rate, $gO_2/m^2/hr$

k = diffusion coefficient ($gO_2/m^2/hr$ at 100% saturation)

S = saturation deficit

A positive diffusion value indicates O_2 diffusion into the water and therefore changes in oxygen concentration are corrected by subtracting $D(gO_2/m^3)$. Values of K between 0.04 and 0.8 were measured in the streams using the floating dome method of Copeland and Duffer (1968) modified by McKellar (1970). In no case did diffusion correction alter the metabolism values by more than 10% of their uncorrected values.

Corrected rate of change data was plotted and areas integrated by counting squares. Nighttime respiration values were averaged and 24 hour respiration (R_{24}) was assumed to equal the average nighttime hourly rate times 24 hours. Gross photosynthesis (PG) was the area above this average R line and net photosynthesis (PNet) equals $P_G - R_{24}$. P/R ratios were calculated as P_G/R_{24}.

Exported organic material and associated Cd were quantified from October 1976 until August 1977. All effluent water from each channel was passed through a four inch ABS plastic pipe into a "T" intersection which contained a motor driven stainless steel mixer blade. Material collected on the end screens was washed into the sampling system daily. Mixed effluent was subsampled from each channel at a rate of 4 liters per day with a peristaltic pump. These subsamples were filtered on to pre-fired Gelman A-E glass fiber filters, dried, weighed, ashed at 450° C and reweighed to obtain ash-free dry weight of exported material. From the length of sampling, the volume of water exported and the volume of the collected subsample, channel export was calculated as grams per channel per day.

Export material was collected off of the end screens for routine Cd analysis. Several grams of material were collected from each screen, blended, and subsampled for analysis. Subsamples were placed in tared crucibles, dried, weighed, ashed at 450° C, and reweighed. The ash material was dissolved with hot HNO_3 and H_2O_2 and then measured as reported in Appendix I.

RESULTS AND DISCUSSION

Overall community metabolism (production and respiration) was measured by the diurnal oxygen method and algal production alone was estimated by the short-term accrual on glass slides described earlier.

Exposure to Cd significantly reduced gross production, net production and respiration at all sampling dates (Fig. 59). Exposure to 5 μg Cd/ℓ resulted in values intermediate between controls and 10 μg Cd/ℓ. Shortly after Cd input was stopped, metabolism values of all the channels converged and were not significantly different from one another. During the period of maximum summer productivity, however, channels which formerly received Cd were slightly depressed compared to the former controls (Fig. 59).

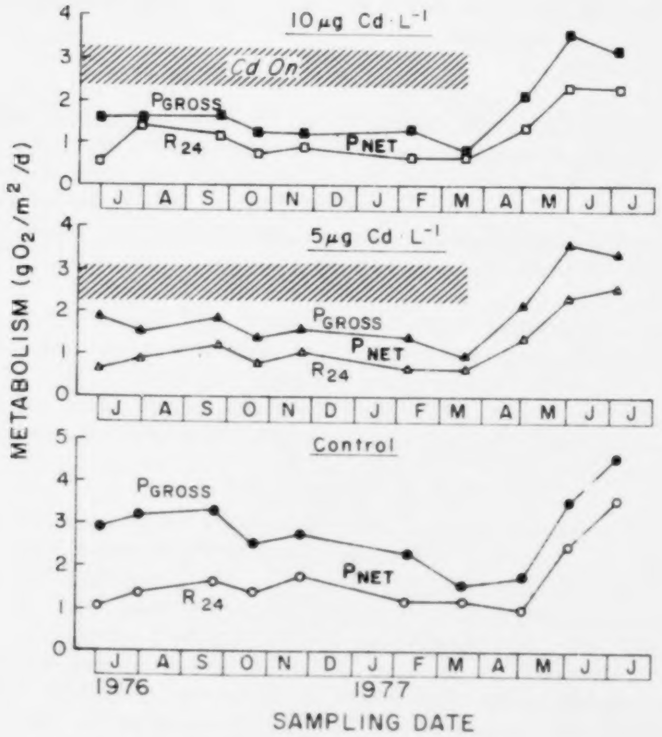


Figure 59. Community metabolism. Gross primary production and respiration with the shaded area representing net production.

Net aufwuchs production and that of its algal component in grams dry weight or live cell volume per square meter per 28-day colonization period (Figs. 60 and 61) should under-estimate the net production of stable communities since populations on glass slides begin colonizing clean slides each sampling period. However, net production estimated in this manner may give qualitative information on seasonal changes as well as a quantitative evaluation of Cd effects.

During the first eight months of Cd input, net aufwuchs production as well as that of the algal component was significantly higher in the control channels. Aufwuchs net production was greatest in the sample collected in June, while algal production was greatest in the August sample (Fig. 60 and 61).

Net production measured by the community method reached a maximum in June and July (Fig. 59). During the second summer, after Cd inputs had been terminated, net aufwuchs and algal production were both near minimum values and yet the community data showed high net production in all treatments. This descrepancy is due to the paucity of vascular plants in the streams during the first summer and their subsequent increase to standing crop dominance by the second summer.

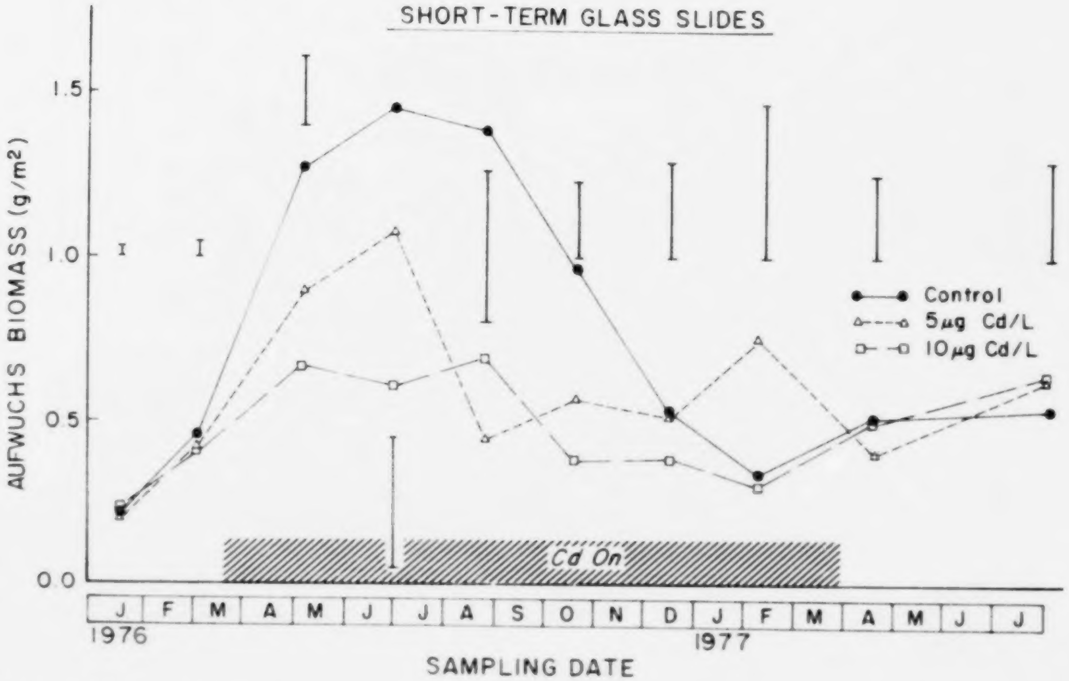


Figure 60. Aufwuchs accrual on short-term glass slides with two standard error confidence intervals indicated.

There was also a greater accumulation of detritus in the control channels. O'Neill et al. (1975) reported that this type of organic accumulation in aquatic systems contributes significantly to the persistence of the ecosystem. Thus the Cd input to the channels may have had long range effects on the succession and stability of the community which developed in the channels. The accumulation of reduced carbon within control channels was due to the greater net productivity in these channels. Experiments on leaf litter decomposition indicated that the microbial decomposer system was inhibited by Cd. (See section XI). Since the inorganic nutrient inputs to the channel microcosm systems was low (see section V) as in other southeastern aquatic ecosystems, the ecosystem stability would be greatly affected by the rate of nutrient remineralization.

Table 27 summarizes the exported organic matter by treatment before and after Cd input. A significantly greater amount of carbon was exported from the control channels than from the treated channels during Cd input. The two treatments were not significantly different with respect to export. After Cd inputs were terminated, significant differences between former treatments disappeared. Day to day export values for all streams were very variable and highly dependent on external energy sources such as rain and wind, and internal changes such as loss of bottom mats and aufwuchs sloughing.

Average Cd levels in export by treatment are presented in Figure 62 on a dry weight basis. For calculation of Cd exported, these values may be converted to an ash-free dry weight basis by multiplying by 0.73 (determined

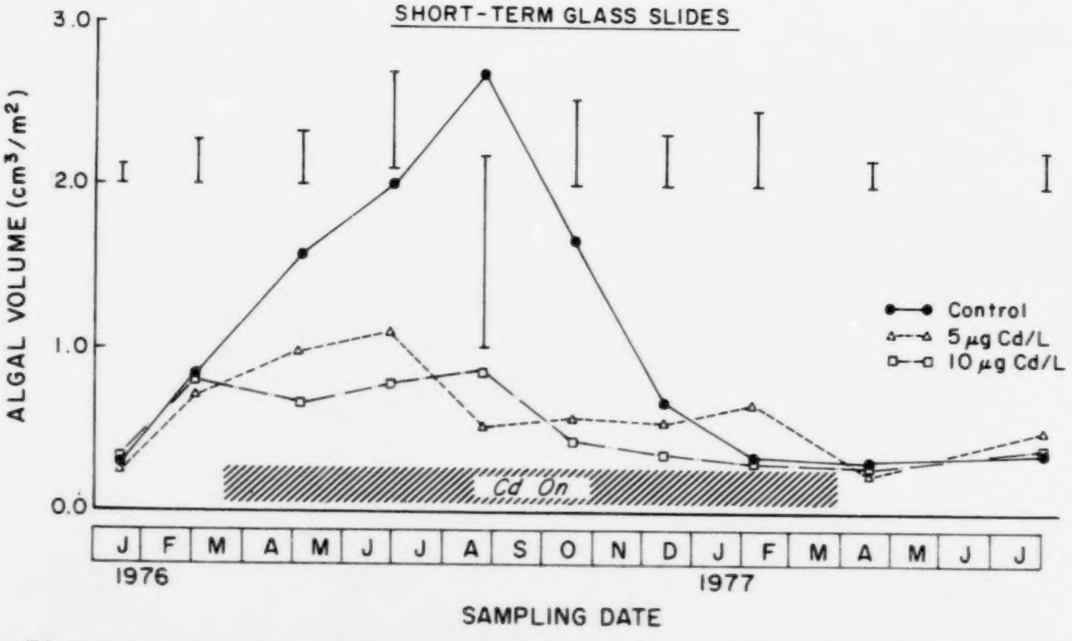


Figure 61. Algal cell volume accrual on short-term glass slides with two standard error confidence intervals indicated.

from 25 export samples with a coefficient of variation of 12%). Great variability on a day to day basis was observed and is related to the variety of sources in the channels that contributed to export. These sources were benthic aufwuchs, wall and glass slide aufwuchs, and macrophytic plants. Cadmium levels in export material was proportional to water Cd concentration levels. Cd export levels decreased to control levels within five months after the inputs were stopped.

Nutrient cycling has been identified as a measurable attribute of the abstract concept of ecosystem stability (Webster et al., 1975) and changes in nutrient cycling have been suggested as measures of changes in community structure (Odum, 1969). The interaction between communities and the elements moving through them can influence species composition, diversity, and stability (Pomeroy, 1975). This approach is attractive because the nutrient dynamics of an aquatic system can be more easily measured than traditional population and community measure. Thus monitoring of changes in nutrient dynamics may be a sensitive system level parameter, reflecting environmental changes. One of the most sensitive biogeochemical cycles has been found to be the nitrogen cycle (R. Todd, personal communication). Unfortunately, the gaseous phases possible in the nitrogen cycle make monitoring of nitrogen fluxes difficult.

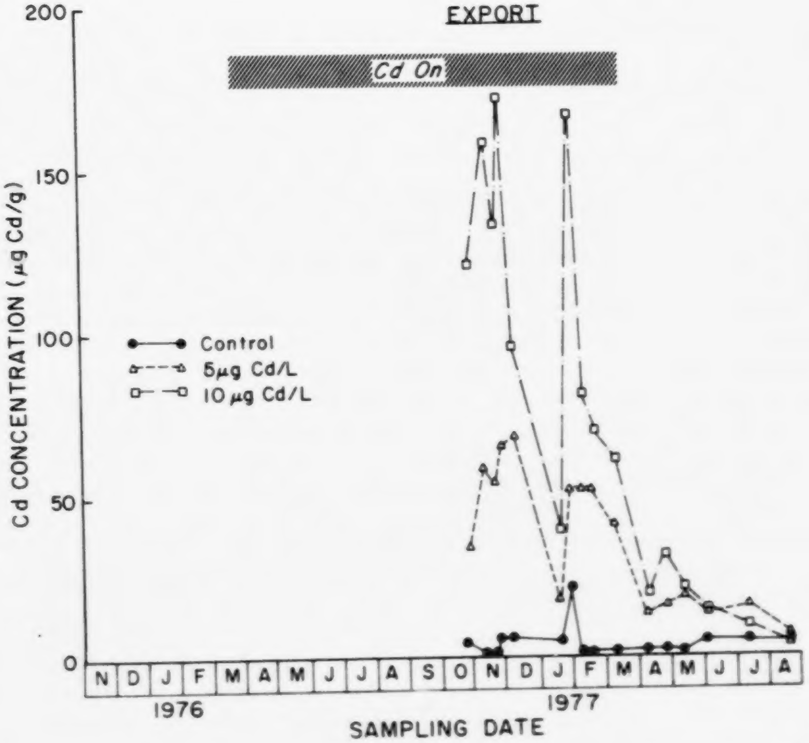


Figure 62. Cadmium concentration in material exported from the channel microcosms.

TABLE 27. SUMMARY OF ORGANIC EXPORT FROM THE CHANNEL MICROCOSMS DURING AND AFTER CADMIUM INPUT. VALUES ARE AVERAGES OF WEEKLY AVERAGES IN GRAMS ASH-FREE DRY WEIGHT $m^{-2} \cdot day^{-1}$

Cadmium On	Control	5 ppb	10 ppb
$\bar{X}$	27.0	20.9	19.4
S.E.	2.1	1.6	1.7
n	21.0	21.0	21.0

Cadmium Off	Control	5 ppb	10 ppb
$\bar{X}$	27.0	25.6	25.1
S.E.	4.7	3.0	4.3
n	8.0	9.0	9.0

We measured upstream and downstream NO_2 + NO_3 levels in each channel (see section V, water chemistry). Ammonia levels were not measured routinely because they were below the detection limits of direct analysis and required concentration, which due to contamination resulted in high variability. The mean NO_2 - NO_3 N concentrations decreased significantly over the length of each channel. There was no significant difference ($P \geq 0.90$) between any of the treatments. While we did observe demonstrable changes in many population, community and system level parameters, these Cd-induced changes were not reflected in changes in NO_2 - NO_3 fluxing in the channels. While this was not a rigorous test of this system level parameter, it does indicate that other measures were more sensitive to Cd stress in our channel microcosms.

A program to measure nitrogen fixation as a system level functional parameter was attempted but because of systematic experimental errors, will not be presented here.

Total orthophosphates (PO_4^{-3}) concentrations did not vary significantly between channels and did not vary between upstream and downstream sampling stations (Table 3). Sulfate (SO_4^{-2}) increased over the length of each channel due to aerial inputs but did not vary significantly due to Cd input (Table 3).

In general, we found nutrient cycling provided little indication of the Cd stresses in the system studied here. Future studies of nutrient cycling as a measure of stress induced changes should include measure of NH_4^+ and K^+.

In many respects the artificial streams used in this study were similar to spring-fed streams which have been extensively studied elsewhere (Odum, 1957a; Odum, 1957b). Water of very low mineral and organic carbon content was introduced constantly over developing plant communities. Many of the species that thrived in the artificial streams are also found in naturally occurring artesian fed streams in the area. Because of this similarity, system measurements made in these channels may be compared to measurements made in natural spring-fed streams and results of Cd input in the artificial streams may be directly extrapolated to some natural systems.

In measuring several natural springs in Florida, Odum (1975a), reported gross production values ranging from 0.7 to 64 g $O_2/m^2/d$ with an average summer value of 17 g $O_2/m^2/d$. Values of gross primary production from the artificial streams is within this range, though they were well below the average value at the end of the Cd study. An obvious difference is that the Florida springs had had many years to develop their communities while the artificial streams had values higher than 3 g $O_2/m^2/d$ after only one year of colonization. The upward slope of all curves during the second summer of water input indicates that successional development was in an early stage and higher metabolism values might be expected in the streams after a longer colonization period.

The autotrophic character of the experimental channels (gross production exceeding respiration) is typical of springs because of a lack of input of organic matter for heterotrophic metabolism (Odum, 1956). However, the result of this system autotrophy must be a combination of net export of organic material and net accrual of biomass. As has been stated elsewhere in this report, the artificial streams were performing both roles with export and biomass accrual both significantly lowered by 5 to 10 μg Cd/ℓ.

The increased metabolism values for treated streams (Fig. 59) after Cd input had stopped are very frustrating because of their correlation with the end of Cd input and the beginning of the summer growth season as observed in the curves for the control channels. Whether this enhanced metabolism reflects a growing adaptation to Cd toxicity or a rebound from the burden of the metal on metabolic processes cannot be established from these data alone. In light of aufwuchs data that showed similar levels of biomass between treatments at the time of Cd shutoff but reduced populations of the algal producers it would appear that no significant adaptation to Cd input was occurring. Different species were being selected for tolerance but their combined effect could not increase productivity to control values in the time range of this study. Yet, in spite of the rigid control by trifling amounts of Cd metal, recovery was almost instantaneous when the toxin was removed at the onset of the prime growing season.

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APPENDIX A

ANALYTICAL TECHNIQUES

The determination of trace levels of Cd in samples of water, biological and geological materials requires a sensitive analytical technique (Paus, 1971). The literature is surfeited with articles devoted to Cd analysis in environmental samples. It is not our purpose to review this literature here. Friberg _et al_. (1975) present a thorough discussion of Cd analytical techniques. Atomic absorption techniques can be used to analyze for trace amounts of Cd in biological material (Harve _et al_., 1973) and is also well suited for the analysis of low levels of Cd usually found in natural waters (Hem, 1972; Ciaccio, 1973; Rattonetti, 1974; APHA, 1975; Briese and Giesy, 1975; Giesy _et al_., 1978) and gives rapid determinations of these levels with high reproducibility involving few interferences. Because of the composition of various biological and geological matrices, there is no standardized methodology suitable for all materials. Because of the obvious importance of accurate Cd analyses for this project, a considerable amount of personnel time and effort has been expended to develop an appropriate set of techniques to sample, prepare and analyze biological and geological materials.

Cadmium determinations were made, using either a Perkin-Elmer Model 306 or Instrumentation Laboratories Model 351 atomic absorption spectrophotometer. Both instruments are equipped with deuterium continuum background correction systems and graphite atomizers for flameless operation. Flame determinations were made using an air-acetylene fuel rich flame with the IL instrument. Flameless determinations were made in normal and interrupted modes using argon as a purge gas with the Perkin-Elmer instrument. We have relied exclusively on flameless techniques for preliminary analyses because of the low background levels present in the organisms introduced to the channels. Later in the project, greater use was made of the more rapid flame determinations as Cd levels increased.

Based on actual analytical results, the sensitivity for the determination of Cd by flame AA in our laboratory is 0.025 μg/ml where sensitivity is defined as that concentration which gives an absorbance reading of 0.0044 (1% A). Our detection limit in the flame mode is 0.005 μg/ml where detection limit is defined as that concentration which gives a signal greater than $2S_x$ above the background noise signal. Both the sensitivity and detection limit vary somewhat in time and with sample matrix.

The sensitivity for flameless atomic absorption is much more variable than that for flame determinations. Since both interrupted or continuous purge gas modes may be used, two sets of sensitivity and detection limits can be calculated for the Perkin-Elmer instrument. Using a continuous flow of argon purge gas, 2.0 pg of Cd produces an absorbance of 0.0044 units. In the interrupted mode, 1.0 pg Cd produces the same value. The interrupted mode of operation is much more variable than continuous and is generally not required. The amount of sample injected into the graphite rod atomizer varies but is usually 10 or 20 µl. Using a 10 µl sample and the continuous purge mode, the sensitivity is 2 X 10^{-4} mg/l in solution. The detection limit varies so much with matrix that it is impossible to give a general value.

The sensitivity and precision of Cd determinations by flameless AA have been optimized for both plant and animal matrices. Drying, charring and atomizing times and temperatures were systematically varied until a program was found which maximized matrix destruction and minimized Cd loss due to volatilization. Based on the results of this preliminary work, a drying of slightly less than 100° C was used for all matrices. A 10 sec drying time was used for 10 µl samples and a 40 sec period for 20 µl samples. Best analytical results were attained when a pyrolysis program of 250-350° C applied for 10-15 sec, regardless of sample size or matrix. The optimum atomization conditions were found to be a temperature of 2000° C applied for 4-5 sec.

All determinations were corrected for reagent blanks and compared against commercially prepared certified standards. Matrix interferences were evaluated in each material analyzed for Cd by the use of internal standards. Background matrix interferences were also checked by determining the absorbance at an adjacent non-absorbing analytical wavelength adjacent to the primary analytical line of 228.8 nm. There is no absorption due to Cd at 226.2 nm but this is near enough to the analytical line so that broad spectrum background absorbances can be determined.

Standard addition curves had the same slope as curves constructed from standards in distilled water, indicating the selected charring and atomization time and temperature regime removed all background interferences for flameless Cd analysis in all matrices. Absorbances determined at the non-absorbing wavelength of 226.2 nm resulted in absorbances of between 0.000 and 0.002, also indicating that background matrix interferences were absent in all matrices.

Sample preparation and analytical procedures were tested by determining Cd in bovine liver (BL) and standard orchard leaves (SOL) supplied by the National Bureau of Standards (NBS). These matrices are analagous to other animal and plant matrices and allow the evaluation of preparatory and analytical techniques. Cadmium concentrations in BL and SOL are below the detection limits of our flame AA techniques. Using flameless methods, however, we measured mean Cd concentrations of 0.31 µg/g dry weight in the BL (NBS certified value is 0.27 ± .03 µg/g) and 0.13 µg/g dry weight in the SOL (NBS certified value is 0.11 ± .02 µg/g).

During the processing of the NBS standards, a number of sources of Cd contamination were discovered. Carry-over in glass and polyethylene reagent and sample bottles is a problem at low Cd levels. Therefore, bottles that have been used for high standards cannot be used to hold lower concentration

Cd standards. No losses from samples or standards occurred during a 24 hr period, but longer periods may cause sorption losses in low Cd standards. Because of these losses, standards are made up from concentrated standards daily. No losses from acidified samples occur over time.

Because of the ubiquity of Cd contamination and the low Cd concentrations we worked with, special care was taken to restrict glassware contamination. The use of marbles in flasks during the digestion process was found to cause contamination and watch glasses were satisfactorily substituted.

Used glassware was immediately rinsed in tap water to remove residual sample or standards and placed in a 1% bath of Contrad (American Hospital Supply Co., McGaw, Ill.) for 24 hr. This wash was followed by several distilled water rinses, a 24 hr. soak in distilled 1% HCl and a minimum of 5 deionized water rinses.

The disposable plastic tips used with Eppendorf pipettes to introduce samples and standards into graphite furnace atomizers vary in Cd contamination from lot to lot, and may introduce considerable error to low level samples. This contamination is reduced by rinsing the plastic tips several times in 10% HNO_3 before use. Even with rinsing, there may be occasional anomalous Cd readings that can be attributed to the plastic tips. The substitution of an automatic Teflon delivery system eliminated this source of contamination.

Reagents have also been found to require special selection and treatment. Hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) are not used in the digestion procedure because chlorides and sulfates are poor matrices for atomic absorption analysis. Perchloric acid ($HClO_4$) is not used because perchloric acid solutions cannot be introduced into graphite rod atomizers for flameless AA determinations. Redistilled reagent grade HNO_3 was found to be free of Cd contamination and satisfactory for use in the digestion procedure. Reagent grade H_2O_2 may be somewhat contaminated with Cd, and corrections must be made from data obtained from reagent blanks.

APPENDIX B

Plants and animals collected from channels during Cd study.

- Phylum Chlorophyta
  - Sub-Phylum Chlorophyceae
    - Order Volvocales
      - Family Chlamydomonadaceae
        - *Chlamydomonas* spp.
    - Order Chlorococcales
      - Family Chlorococcaceae
        - *Characium* sp.
        - *Chlorococcum humicola* (Naegeli) Rabenhorst
      - Family Oocystaceae
        - *Eremosphaera viridia* DeBary
      - Family Scendesmaceae
        - *Coelastrum* sp.
        - *Scenedesmus acutiformis* Schroeder
        - *Scenedesmus* sp.
    - Order Ulotrichales
      - Family Ulotrichaceae
        - *Hormidium subtile* (Koetzing) Heering
        - *Geminella turfosa* (Skuja) Ramanathan
      - Family Microsporaceae
        - *Microspora pachyderma* (Wille) Lagerheim
    - Order Chaetophorales
      - Family Chaetophoraceae
        - *Microthamnion strictissimum* Rabenhorst
        - *Stigeoclonium elongatum* (Hassall) Kuetzing
    - Order Oedongoniales
      - Family Oedogoniaceae
        - *Oedogonium* sp.
    - Order Zygnematales
      - Family Zygnemataceae
        - *Mougeotia* spp.
      - Family Desmidiaceae
        - *Cosmarium asphaerosphorum* Nordstedt
        - *C. laeve* var. *septentrionale* Wille
        - *C. pseudoconnatum* var. *ornatum* Allorge
        - *C. viride* var. *minor* West
        - *Euastrum* sp.
        - *Spaerozosma excavata* Ralfs
        - *Spondylosium planum* West and West
        - *Staurastrum alternans* Brebisson

- Phylum Euglenophyta
  - Order Euglenales
    - Family Euglenaceae
      - *Euglena* *mutabilis* Schmitz

- Phylum Chrysophyta
  - Sub-Phylum Chrysophyceae
    - Order Chromulinales
      - Family Chromulinaceae
        - *Chromulina* *pseudonebulosa* Pascher
  - Sub-Phylum Bacillariophyceae
    - Order Pennales
      - Family Naviculaceae
        - *Navicula* *notha* Wallace
        - *Pinnularia* sp.
      - Family Fragilariaceae
        - *Synedra* sp.
      - Family Epithemiaceae
        - *Rhopalodia* sp.

- Phylum Cyanophyta
  - Order Chroococcales
    - Family Chroococcaceae
      - *Merismopedia* *punctata* Meyer
  - Order Oscillatoriales
    - Family Oscillatoriaceae
      - *Oscillatoria* *geminata* Meneghini
  - Order Nostocales
    - Family Nostocaceae
      - *Anabaena* sp.
    - Family Scytonemataceae
      - *Microchaete* sp.
    - Family Rivulariaceae
      - *Calothrix* *parietina* (Naegeli) Thuret

- Phylum Pyrrhopyta
  - Class Dinophyceae
    - Family Glenodiniaceae
      - *Glenodinium* sp.

- Phylum Spermatophyta
  - Family Callitrichaceae
    - *Callitriche* *heterophylla*
  - Family Juncaceae
    - *Juncus* *acuminatus*
    - *Juncus* *diffusissimus*
  - Family Lentibulariaceae
    - *Utricularia* *biflora*
  - Family Poaceae
    - *Agrostis* *hyemalis*
  - Family Polygonaceae
    - *Polyganum* *hydropiperoides*

Family Scrophulariacease
Gratiola virginiana
Family Typhaceae
Typha latifolia

	Collection Method	
	plate sample	bottom sample
Phylum Arthropoda		
Class Insecta		
Order Ephemeroptera		
Family Baetidae		
Callibaetis sp.	X	X
Baetis sp.	X	
Family Caenidae		
Caenis sp.	X	X
Family Leptophlebiidae		
Paraliptophebia sp.	X	
Order Odonata		
Suborder Anisoptera		
Family Libellulidae		
Pantala hymenaea	X	X
Erythrodiplax minusula	X	X
Pachydiplax longipennis		X
Erythemis sp.		X
Celithemis fasciata		X
Suborder Zygoptera		
Family Coenagrionidae		
Argia sp.	X	X
Ischnura sp.	X	X
Order Hemiptera		
Family Mesoveliidae		
Mesovelia sp.		X
Family Hebridae		
Merragta sp.		X
Family Gerridae		
Gerris sp.	X	X
Family Veliidae		
Microvelia sp.	X	X
Family Navcoridae		
Pelocoris femoratus	X	
Family Nepidae		
Ranatra sp.	X	
Family Notonectidae		
Notonecta indica	X	X
Burnoa seimitra	X	
Family Corixidae		
Hesperocorixa sp.	X	X

	Collection Method	
	plate sample	bottom sample
Order Trichoptera		
Family Hydroptilidae		
Oxyethira sp.		
Family Psychomyiidae		
Polycentropus sp.		
Order Lepdioptera		
Family Pyralidae		
Pyrausta sp.		X
Nymphula sp.		X
Order Coleoptera		
Family Gyrinidae		
Gyrinus sp.		X
Family Noteridae		
Hydrocanthus iricolor	X	X
Family Haliplidae		
Haliplus sp.	X	
Family Dytiscidae		
Bidessus sp.	X	X
Hydroporus sp.	X	X
Laccophilus sp.	X	X
Family Hydrophilidac		
Berosus sp.	X	X
Enochrus sp.		X
Tropisternus sp.		X
Family Elmidae		
Stenelmis sp.	X	X
Family Dryopidae		
Helichus sp.		X
Order Diptera		
Family Tipulidae		
Helius		X
Limonia sp.		X
*Family Chironomidae		
Subfamily Chironominae	X	X
Chironomus sp.		
Cladotanytarsus sp.		
Cryptrochironomus sp.		
Polypedilum sp.		
Rheotanytarsus sp.		
Tanytarsus sp.		
Subfamily Orthocladiinae	X	X
Cardiocladius sp.		
Cricotopus sp.		
Corynoneura sp.		
Thienemanniella sp.		

	Collection Method	
	plate sample	bottom sample
Subfamily Pelopiinae	X	X
Ablabesmyia ornata		
A. peleensis		
Family Ceratopogonidae		
Bezzia or *Prubezzia sp.*	X	X
Dasyhelea sp.	X	X
Family Tabanidae		
Chrysopy sp.		X
Tabanus sp.		X
Phylum Plathelminthes		
Class Turbellaria		X
Phylum Annelida		
Class Hirudinae		X
Class Oligochaeta		
Order Prosopora		
Family Lumbriculidae		X
Order Pleisiopora		
Family Naididae		
Pristina sp.	X	
Phylum Mollusca		
Class Gastropoda		
Subclass Pulmonata		
Family Physidae		
Physa sp.	X	

*only Identified from limited Number of Samples.

- Phylum Protozoa
  - Subphylum Plasmodroma
    - Class Mastigophora
      - Subclass Phytomastigina
        - Order Chrysomonadina
          - Suborder Eucrysomonadina
            - Family Chromulinidae
              - *Chromulina* *sp*.
              - *Oikomonas* *sp*.
              - *Crysamveba* *sp*.
              - *Mallomonas* *sp*.
            - Family Ochromonadidae
              - *Ochromonas* *sp*.
          - Suborder Rhizochrysidina
              - *Rhizochrysis* *sp*.
        - Order Cryptomonadida
          - Suborder Eucryptomonadina
            - Family Cryptomonadidae
              - *Cryptomonas* *sp*.
              - *Cyathomonas* *truncata*
        - Order Phytomonadida
            - Family Chlamydomonadidiae
              - *Chlamydomonas* *sp*.
        - Order Eyglemoidida
            - Family Euglenidae
              - *Euglena* *spp*.
            - Family Astasiidae
              - *Astasia* *sp*.
            - Family Anisonemidae
              - *Anisonema* *spp*.
              - *Peranema* *sp*.
      - Subclass Zoomatigia
        - Order Rhizomastigida
            - Family Mastigamvebidae
              - *Mastigamoeba* *sp*.
    - Class Sarcodena
      - Subclass Rhyopoda
        - Order Proteomyxida
            - Family Vampyrellidae
              - *Nuclearia* *sp*.
              - *Vampyrella* *sp*.
              - *Hyalodiscus* *sp*.
              - *Reticulomyxa* *sp*.
        - Order Amoebida
            - Family Amoebidae
              - *Amoeba* *proteus*
              - *Amoeba* *discoides*
              - *Amoeba* *dubia*
              - *Amoeba* *spp*.

Vahlkampfia *sp.*
Hartmannella *sp.*
Order Testacida
Family Arcellidae
Arcella *vulgaris*
Family Difflugiidae
Difflugia *urcevlata*
Difflugia *corona*
Difflugia *globosa*
Difflugia *lobostoma*
Centropyxis *sp.*
Family Euglyphidae
Euglypha *sp.*
Subclass Actinopoda
Order Heliozoida
Family Actinophryidae
Actinophrys *sol*
Actinosphoerium *sp.*
Subphylum Ciliophora
Class Ciliata
Subclass Holotricha
Order Gymnostomatida
Family Holophryidae
Holophrya *sp.*
Lacrymaria *sp.*
Family Colepidae
Coleps *sp.*
Family Tracheliidae
Dileptus *spp.*
Family Loxodidae
Loxodes *sp.*
Family Chlamydodontidae
Chilodonella *sp.*
Order Trichostomatida
Family Colpodidae
Colpoda *sp.*
Order Hymenostomatida
Family Tetrahymenidae
Colpidium *sp.*
Family Parameciidae
Paramecium *bursaria*
Paramecium *caudatum*
Paramecium *aurelia*
Paramecium *sp.*
Subclass Spirotricha
Order Heterotrichida
Family Spirostomatidae
Spirostomum *spp.*
Blepharisma *sp.*
Family Stentoridae
Stentor *sp.*

Order Oligotrichida
Family Halteriidae
Halteria sp.
Order Hypotrichida
Family Oxytrichidae
Uroleptus sp.
Urostyla sp.
Family Euplotidae
Euplotes spp.
Subclass Peritricha
Order Peritrichida
Vorticella sp.

APPENDIX C

Published information supported in part by Interagency agreement EX-76C-09-0819 between the U.S. Environmental protection agency and U.S. Department of Energy.

1) Giesy, J. P., G. J. Leversee and D. R. Williams. 1977. Effects of naturally occurring aquatic organic fractions on cadmium toxicity to *Simocephalus serrulatus* (Daphnidae) and *Gambusia affinis* (Poeciliidae). *Water Res.* 11:1013-1020.

2) Williams, D. R. and J. P. Giesy. 1978. Relative importance of food and water sources to cadmium uptake by *Gambusia affinis* (Poeciliidae). *Environ. Res.* 16:326-332.

3) Giesy, J. P. 1978. Cadmium inhibition of leaf decomposition in an aquatic microcosm. *Chemosphere* 6:467-475.

4) Thorp, J. H., J. P. Giesy and S. A. Wineriter. 1978. Effects of chronic cadmium exposure on crayfish survival, growth, and tolerance to elevated temperatures. *Arch. Environ. Cont. Toxicol.* (Submitted).